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Page 82, abstract 2, name, *read* A. H. Gilbert *for* G. H. Gilbert.

Page 84, line 6, *read* cowpea roots in 8 hours and within pineapple roots in 24 hours *for* roots in 8 hours.

Page 92, abstract 1, line 5, *read* externally *for* internally.

Page 94, abstract 1, line 12, *omit* in case of Verticillium wilt.

Page 108, line 2, *read* acetone *for* acetate.

Page 144, Table 1, column 1, last line, *read* (Fr.) Ces. *for* Sor.; column 2, *read* ..... *for* 5th do and 6th do; column 3, *read* ..... *for* 2d do and 3d do; column 4, *read* ..... *for* each do.

Page 147, paragraph 1, line 13, *read* headblight *for* hedblight.

Page 163, title, *read* Disease *for* Diease.

Page 171, paragraph 4, line 10, *read Sclerotium for Sclerotinia.*

Page 172, footnote, *read* D. T. Killough *for* D. J. Killough.

Page 173, paragraph 1, line 1, *read* reveal *for* reval.

Page 318, line 1, *read* bowed *for* booked.

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Page 443, paragraph 2, line 9, *read* re-sorted *for* restored.

Page 479, line 13, *after* Virginia *add* and Illinois as reported by Anderson (Phytopathology **14**: 31. 1924).

Page 565, line 9, *read* Mc Rae *for* McRea.

Page 570, line 6, *read* neighbor *for* neighboring.

Page 572, paragraph 2, line 9, *read* or *for* of.

Page 624, paragraph 1, line 13, *read* 9, *a, for* 10, *a.*

Page 682, paragraph 1, line 1, *read* Turkey (*T. vulgare*) *for* Velvet Don (*T. durum*); figure legend, *read* Turkey (*T. vulgare*) *for* Velvet Don (*T. durum*); paragraph 2, line 1, *read* Velvet Don (*T. durum*) for Turkey (*T. vulgare*).

Page 683, figure 3 legend, *read* Velvet Don (*T. durum*) *for* Turkey (*T. vulgare*).

Page 742, paragraph 2, line 11, *read* and then *for* and the.

Page 872, paragraph 3, line 2, *read garryana for garyana.*

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Plate XXXIV (opp. page 932), *read* 600 *for* 1600.

580.5-27

Page 965, line 6, *read* very *for* vary.

Page 966, paragraph 5, line 1, *read* Sclerosporas *for* Sclerospora.

Page 967, line 8, *read* far *for* fas.

Page 972, last line, *read* Mycosphaerella *for* Myosphaerella.

Page 973, paragraph 7, line 2, *change* blue to green.

Page 1019, paragraph 3, line 2, *read* Sirexcipulina *for Sirex cipulina.*

Page 1021 line 4, *read* apothecial *for* apothetical.

Page 1042, paragraph 1, line 5, *after* infection *insert* of.

Pages 1033, 1035, running heads, *after* beet *add* curly top.

December issue, first printed page, line 4 under Associate Editors, *after* Station *insert* comma.

Page 1073, paragraph 3, line 4, *read* attached *for* atached.

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Page 1135, abstract, title, *read terrestris for terrestria.*

Page 1136, line 3, *read* terrestris *for* terrestria.

Page 1141, abstract 2, line 4, *read* power to infect and produce severe symptoms *for* infection power and the development of severe symptoms.

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THE RELATION OF WEATHER TO THE DEVELOPMENT OF STEM RUST IN THE MISSISSIPPI VALLEY[1]

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INTRODUCTION

It is well known that destructive epidemics of stem rust, *Puccinia graminis* Pers., occur in the spring wheat area during certain seasons, while other seasons are comparatively free from rust. Obviously it is important to know the reasons for this fluctuation. As the development of epidemics was studied year after year, it has become more and more evident that many interdependent factors are involved. In the Northern States all phases of the life cycle of the rust organism must be considered. The development of rust throughout the entire Mississippi Valley also seems to have a bearing on the problem. Seasonal weather conditions appear to be an important factor, but until recently our information on this subject has been based on more or less isolated observations.

Controlled experiments (15, 19, 20, 41, 44, 45, 46, 47, and 58) have indicated that temperature and moisture conditions profoundly influence the germination of teliospores and sporidia, the germination and longevity of urediniospores, the germination of aeciospores, and the rate of development of aecial and uredinial infection. Field observations have been made, but there is no unanimity of opinion. Certain workers (6 and 24) believe low temperatures favor the development of epidemics. Some investigators (14, 33, 58 and 64) have presented evidence that warm growing seasons are correlated with heavy rust development; others (2 and 67) are inclined to believe that rainfall is the major factor; still others (11, 12, and 54) suggest that high humidity and dew are more important than rainfall. No

[1] The data presented in this paper were obtained in cooperative investigations by the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Agriculture of the University of Minnesota; and submitted to the graduate faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted June 13, 1927.

[2] The writer gratefully acknowledges his indebtedness to Dr. E. C. Stakman for access to unpublished papers on the epidemiology of stem rust, and for encouragement and helpful criticism during the progress of the investigation.

one, however, has attempted to present a perspective of the relation of weather to all of the different phases of the development of epidemics as they occur in the Mississippi Valley.

STATEMENT OF THE PROBLEM

A brief sketch of the way stem rust develops in the Mississippi Valley seems necessary as a basis for a discussion of the rôle played by the weather. Apparently no two epidemics develop in exactly the same manner. But, although they vary considerably from year to year, the general sequence of events is usually the same. The field studies of Stakman and others[3] indicate that the cycle of the rust is different in the Southern States from that in the Central and Northern States. This is represented diagrammatically in figure 1.

In southern Texas the uredinial stage of the rust increases slowly from October to January. It then decreases in amount in January and February, reaching low ebb in March. It steadily increases from April and May until harvest. In northern Texas and Oklahoma the cycle is approximately the same except that the rust as a rule probably does not persist through March and April, and the development in the spring usually does not begin until May.

In northern Kansas and Nebraska, there is no conclusive evidence of the persistence of the uredinial stage through the winter and spring months. Barberries become infected late in April and early in May, and spread rust to grain and grasses in May. Otherwise the cycle is similar to that in northern Texas and Oklahoma.

The germination of teliospores and the infection of barberries are the first indications of an approaching epidemic in the spring wheat area. The teliospores begin to germinate early in April, and pycnia usually appear on barberries in late April and early May. Usually stem rust is spreading from barberries to grasses and grain during the last two weeks in May. Toward the middle of June primary infection begins to appear in the wheat fields throughout the southern part of the area, regardless of their proximity to barberries. In most years there is a general sprinkling of primary infection on susceptible grains as far north as the Canadian border by the first of July. From then until harvest successive generations of rust develop at intervals of from five to ten days. Most of the damage caused by

[3] These studies have been made by the Office of Cereal Crops and Diseases, U. S. Department of Agriculture, under the direction of Dr. E. C. Stakman, each year for the past eight years. At present they are in the form of unpublished progress reports. The writer has had access to these reports because he assisted in the work for the past five years. Much of the material presented in this paper was obtained during that time.

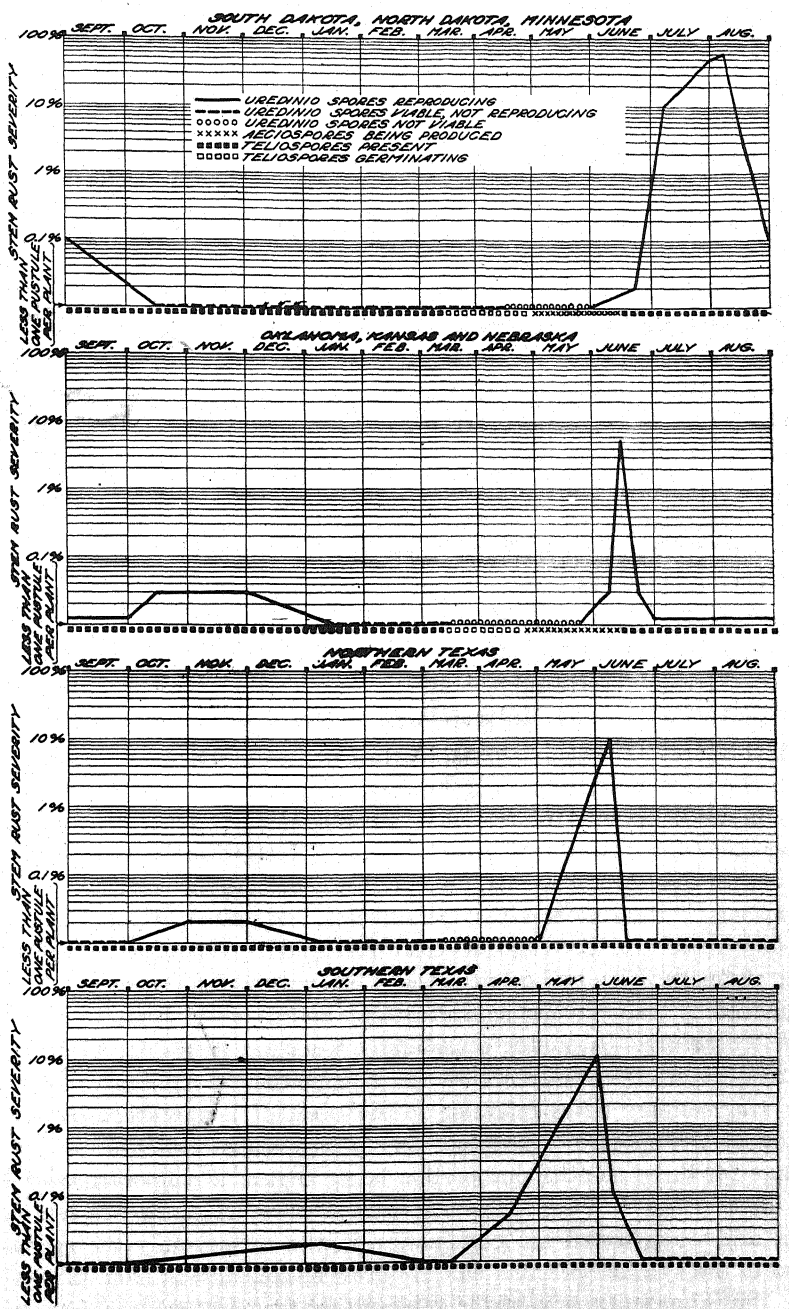


FIG. 1. Diagram of the approximate development of the different phases of the life cycle of stem rust in the average year in the western Mississippi Valley.

stem rust occurs shortly before harvest, during the period when the grain is filling. In South Dakota and southern Minnesota the wheat is harvested late in July. In the northern part of the area the harvest continues through August. Teliospores remain viable until the next season, but the uredinial stage does not persist.

It is apparent from this brief sketch that an attempt to analyze the relation of weather to the development of stem rust in the Mississippi Valley must take into consideration the effect of weather on all of the phases in the life cycle of the rust; on the development of rust in different regions and in different seasons of the year; and on the severity of epidemics from year to year. Finally, it must be recognized that the effect of weather on one phase of the life cycle may profoundly influence the development of another phase and, likewise, that the regional, seasonal and yearly developments of rust may be interdependent.

In the present work, controlled experiments have been largely confined to the determination of the factors influencing the maturation of teliospores and the infection of barberries. Field observations and comparative weather data also are given in an attempt to present a perspective and lay a foundation for further work.

MATURITY AND GERMINATION OF TELIOSPORES AND SPORIDIA

Maturation of Teliospores

Teliospores of *Puccinia graminis* rarely, if ever, germinate immediately after they are produced. This seasonal response was noted by De Bary (16) in 1865. It is extremely important in the economy of the pathogene, since, otherwise, teliospores produced in the North would germinate in the fall rather than in the spring, and eliminate themselves from the life cycle of the rust. Because this rest period is so important in the function of teliospores, several investigators have attempted to determine the factors affecting maturation and germination. The rest period conceivably may be influenced by temperature, moisture, light, and chemical stimuli.

Temperature.—Although considerable work has been done to determine the temperature requirements for the germination of teliospores, there are only fragmentary notes referring to the effect of temperature on the rest period of the spores. McAlpine (35) placed badly rusted straw in cold storage for three months, one portion being kept at a temperature of 4° C. and another at −18° C. A third portion of the same straw was left in the open, and when tested for germination in the spring, only the spores exposed to the weather germinated. He drew no conclusions from this experiment, but apparently was of the opinion that temperature was not a major factor. Klebahn (32), after testing the germination of teliospores of *P.*

graminis, stored indoors and outdoors under various conditions, concluded that winter cold was not necessary but seemed to have an accelerating effect on germination. Thiel and Weiss (63) were unable to shorten the rest period of teliospores by exposure to −10° C. and to alternate freezing and thawing in December.

During the winters 1924–28 the writer has subjected teliospores to different constant temperatures and relative humidities to determine their effect on the maturation period. In one experiment, teliospore material of *P. graminis secalis, P. graminis tritici,* and *P. graminis avenae* was collected in September, 1923, and stored at three different temperatures, 10°, 22°, and 35° C., in constant humidities. Germination tests were made with this material in September, December, and April, by floating the spores on the water at 18° C. Only the *secalis* strain germinated. This material was on *Agropyron repens.* As indicated in table 1, the spores germinated abundantly when stored at 10° and 22° C., but none of the spores kept at 35° C. seemed to be viable. The experiment was repeated with spores of *P. graminis tritici* in the winter of 1924–1925. In this experiment an additional series was kept at 0° C., making four constant temperatures. The results of this test, which are also included in table 1, were essentially in agreement with those obtained with the *secalis* strain. The percentage of germination was low in all lots except that stored at 35° C., in which none of the spores germinated. In both winters, spores from the same collections were stored outdoors as checks on the germination of those kept at constant temperatures. These spores germinated at the same time and approximately as well as those stored indoors.

To test the effect of alternate freezing and thawing on the period of dormancy, dry and wet spores of *Puccinia graminis secalis* were alternately frozen and thawed at approximately 10-day intervals throughout the winter of 1923. None of these spores germinated until spring, nor did any of the wet spores germinate even at that time. In this connection it may be noted that the writer has on several occasions confirmed the observations of Melhus *et al* (41) that spores lying on the ground germinate very sparingly, if at all, in the spring. In April the dry spores which had been alternately frozen and thawed germinated as well as, but no better than, two other lots of dry spores which had been kept as checks, one indoors and the other outdoors.

These results indicate that a high temperature, 35° C., for long periods is injurious to teliospores. Otherwise, temperature does not seem to be a major factor in determining their maturation.

Moisture.—The effect of drying, and especially alternate wetting (soaking) and drying, on the maturation of teliospores of various rusts has been

TABLE 1.—*The effect of different constant temperatures and humidities on the rest period of teliospores of Puccinia graminis secalis and Puccinia graminis tritici as indicated by germination tests*

Stored in the dark at		Average germination at 18° C. in duplicate trials[a]						
		Puccinia graminis secalis stored in Sept., 1923, and germinated				*Puccinia graminis tritici* stored in Sept., 1924, and germinated		
Temperature, degrees C.	Relative humidity, per cent	Sept. 29, 1923	Dec. 29, 1923	Apr. 18, 1924	Apr. 24, 1924	Sept. 18, 1924	Apr. 2, 1925	Apr. 24, 1925
0–2	0	–	–	–	–	0	tr	+
	20	–	–	–	–	0	0	+
	40	–	–	–	–	0	+	0
	60	–	–	–	–	0	+	tr
	80	–	–	–	–	0	0	tr
10–15	0	0	0	+	+	0	0	tr
	20	0	0	+	+	0	tr	tr
	40	0	0	+	+	0	0	+
	60	0	0	+	+	0	0	+
	80	0	0	+	++	0	0	tr
22	0	0	0	+	0	0	0	tr
	20	0	0	0	+	0	tr	0
	40	0	0	+	++	0	+	0
	60	0	0	+	+	0	0	0
	80	0	0	0	tr	0	0	0
35	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0

[a] 0 – no germination.
tr – few spores germinating.
+ – at least one clump of spores germinating abundantly.
++ – more than 10 per cent germination.

noted repeatedly (37). Klebahn (32) made controlled experiments with *P. graminis*. In the winter of 1911, he stored a collection of spores in three different ways: indoors in a dry room; in the open exposed to cold and dampness, but not to rain; and indoors alternately wet and dried at three-day intervals.

Those spores which were wet and dried at frequent intervals germinated in the spring, while the others did not. In 1912 he repeated the experiments, using running water to wet the spores. In this experiment the spores which were alternately wet and dried germinated well in January, but less than 1 per cent germinated when they were kept in a warm dry room. He concluded that the most important factor for germination was saturation and drying. The writer was unable to confirm these results with teliospores collected in the spring wheat area in September, 1924. A part of this material, on *Agropyron repens,* was kept at 5° C. and alternately wet and dried at approximately weekly intervals throughout the winter. Repeated germination tests with both the check and treated material failed until March and April, when clumps of spores germinated in both lots. There was no measurable difference between the germination in the check and the treated material. The experiment was repeated in 1925, but all the material used failed to germinate in the spring.

The experiments already described to determine the effect of temperature were also adapted to determine the effect of constant humidities on the rest period of teliospores. At each of the four temperatures, the straws bearing the spores were placed in sealed Mason jars which contained sulfuric acid, or saturated salt solutions to fix the humidity. In 1923 different concentrations of sulfuric acid were used, as suggested by Wilson (66). In 1924 saturated salt solutions were used to control the humidity, according to the method described by Headlee (26). Five jars were used at each temperature and the relative humidity in them was adjusted to approximately 0, 20, 40, 60, and 80 per cent. The results are given in table 1. It is apparent that differences in relative humidity had no appreciable effect on the rest period or viability of teliospores.

In 1923 a similar series of five sealed jars was set outdoors. The material in these jars reacted quite similarly to that in the jars kept indoors. Small percentages of spores germinated in the spring, but there were no great differences between the amount of germination of the material kept at different humidities. The response obtained in these experiments was different from that obtained by Klebahn. This may have been due to the material used, or to the conditions of the experiment. However, it is clear that alternate wetting and drying is not always necessary for the maturation of spores. Under some circumstances it may shorten the period of

dormancy, but the evidence of this does not seem clear cut enough to consider alternate wetting and drying as a limiting factor in the maturation of teliospores.

Light.—In the experiments described above, in which the spore material was kept at constant temperatures and humidities, the spores were stored in the dark. The fact that these spores germinated proves that light is not necessary for maturation, but there was no check material kept in the light to compare with that stored in the dark. Therefore, two additional experiments were made to determine the effect of this factor. In one case, teliospores were exposed constantly to artificial light for two months early in the winter. This treatment failed to shorten the rest period. The second experiment was begun in September, 1923. Straw of *Agropyron repens* bearing fresh teliospores was placed in four glass jars; two of these were painted black, and two were left unpainted. One of the blackened and one of the unpainted jars were sealed; the other two were left open, but inverted over a wire screen in such a way as to allow air to enter but to exclude direct sunlight from the blackened jar. Germination tests were made in April of the following spring. Clumps of spores taken from the material in all of the jars germinated vigorously. There was no measurable difference between the percentages of germinating spores when kept in the dark and when kept in the light.

Chemical stimuli.—In 1920, Thiel and Weiss (63) offered two hypotheses to account for the rest period: after-ripening of the spore contents, and relative impermeability of the spore wall to water. They attempted to shorten the rest period by applying methods which had been effective in shortening the rest period of seeds. Keeping the spores at constant low temperatures and subsequent freezing and thawing failed to stimulate them. Lipoid solvents and various acids also were used in an attempt to modify the permeability of the spore wall, or to affect the enzymic changes occurring within the spore protoplasm and to increase its hydration capacity. All of the lipoid solvents and acids which were tested were ineffective in stimulating germination, except citric acid. Teliospores which were exposed to chloroform vapor for one minute, followed by a fifteen-minute immersion in 1 per cent citric acid, germinated in December, when check material would not germinate. The treatment was repeated twice in December, twice in January and once in February with consistent results. They were inclined to believe that citric acid acted as a specific stimulant, because other acids did not produce a similar effect.

The writer has attempted on several occasions to use this method of stimulating teliospore germination, but it has never been successful. Several modifications of the method also have been tried without success. In

addition, numerous other chemicals were used in attempts to shorten the rest period. The following are a few of the chemicals tested: straw extracts, and extracts of decayed and living plant tissue in various concentrations; benzaldehyde, salicylaldehyde, citraldehyde, several alcohols; the sixteen essential oils used by Noble (43) to stimulate the spores of *Urocystis tritici;* ethylene gas; and ethylene chlorohydrin, which Denny (17) found to be an effective stimulant for breaking the rest period of potatoes.

Several nitrate salts and a series of salt solutions (13) ranging in pH value from 5.2 to 8.0 also were tried in several concentrations. Germination tests were made also with spores in water containing growing roots and on the surface of distilled water under bell jars beside growing plants. None of the methods or substances tried appeared to influence markedly the rest period. There was some evidence of increased germination when certain of the chemicals were applied to matured teliospores. This will be discussed later in connection with the factors affecting the germination of mature spores.

It is quite possible that a chemical method of shortening the rest period of teliospores will be found in the near future, but the writer is inclined to believe that chemical stimuli do not play an important part in the germination of teliospores in nature. There is a possibility that material such as the products of decaying straw, enzymes produced by saprophytes on the straw, or volatile oils from the spring flora may influence the rest period in nature. But teliospores usually germinate on straw and not in the soil or on the barberry. Therefore the presence of host tissues would not be expected to stimulate them in the same manner as it does the spores of *Botrytis cinerea* Pers. (9) or *Urocystis tritici* Koern. (43), for example. Moreover, teliospores which matured under controlled conditions germinated when these factors were not operating, and immature spores did not germinate when many chemical stimuli were present. Furthermore, in the writer's cultures a few spores have matured in December and they often mature in February, before many external stimuli have had a chance to operate.

Time.—Reference already has been made to the fact that teliospores of *Puccinia graminis* which are formed in the fall usually do not germinate until spring.

In the past five years at St. Paul, Minnesota, teliospores have begun to respond to favorable conditions for germination in February. When tested in distilled water at 18° C., spores collected in the vicinity of University Farm, St. Paul, Minn., germinated on the following dates:

1923—February 26[4]—on *Agropyron repens*
1924—January 18 —on *Poa pratensis*

[4] Tests in 1923 were made by D. L. Bailey, formerly agent in the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

1925—January 21 —on *Triticum vulgare*
1926—March 3 —on *Agropyron repens*
1927—February 25 —on *Agropyron repens*

On these dates only a few spores in several hundred germinated. Maximum germination did not occur until April. This is approximately the season recorded by other workers. Dietel (19) found that teliospores of *P. graminis* began to germinate in March in central Germany. In Australia, according to McAlpine (35), "It is only after a considerable rest that they will germinate and they start on warm days about the end of September, which is the beginning of our spring. This may continue to the warmer days of October, almost or entirely ceasing in a cold spell, and even until November germination continues if conditions are favorable; but, although numerous trials have been made, no germination has occurred outside of these months." Eriksson (20) showed that in Stockholm, Sweden, these spores germinated under natural conditions in April and May. At Madison, Wisconsin, and Ames, Iowa, Melhus *et al* (41) also found that, under natural conditions, teliospores of stem rust become viable in April.

This apparent dormancy of teliospores throughout the winter months suggests that time is necessary in order to enable certain changes to take place in the spore, possibly changes in the physical state of the protoplasm which transform it from a gel to a hydrosol as suggested by Price (50). The mere fact that spores formed in the fall failed to germinate until spring is not conclusive evidence that time is the most important factor, unless it can be shown that no external stimulus has acted in the intervening period.

However, two kinds of evidence indicate that time is an important factor. In the first place, it has been shown that teliospores do not readily respond to external stimuli such as temperature, moisture, light, and chemicals. In fact, most of the efforts which have been made to induce spores to germinate before February have failed. Secondly, teliospores kept under controlled conditions would not germinate in the winter, but germinated readily in the spring. This was demonstrated in two separate seasons. In these experiments, unknown external stimuli could hardly account for the difference in the spores in the winter and spring, because the material was kept at constant temperature and moisture in the absence of light and the spores germinated in distilled water. All of the evidence at hand points strongly toward the conclusion that teliospores have a true rest period which carries them through the fall and early winter months during which environmental conditions apparently have little effect on the spores.

Germination of Mature Teliospores

After teliospores have matured, their germination and the production of sporidia may be influenced by humidity and temperature. Light and the reaction of the medium do not seem to be important.

Moisture.—Melhus *et al* (41) compared the germination of teliospores in water and in atmospheres of different relative humidities. They agree with Blackman, Magnus, and Kunkel that only abnormal germ tubes, and seldom sporidia, are formed under water. Abundant germination and sporidial formation occurred in a saturated atmosphere, but only a few sporidia were produced at 95 per cent relative humidity, and none were formed in less humid atmospheres. These experiments indicate that in nature germination and liberation of sporidia must take place during rains.

Temperature.—According to Dietel (19), the cardinal temperatures for the production of sporidia are 9.5° C., 22° C., and 23° C. Melhus *et al* (41) essentially confirmed these results, but in their experiments the germination was best at 20° C. and growth and the production of sporidia were barely noticeable at 5° C. In the writer's experiments the spores germinated well at 12–18° C. but they germinated most consistently at 18° C.

Light.—To determine the relation of light to germination, spores of *P. graminis secalis* and *P. graminis tritici* were placed in full sunlight, diffused light, and darkness, at approximately 18° C. The tests were made with the spores floating on distilled water in sealed syracuse dishes. Some difficulty was experienced in regulating the temperature, but in cases where the water in the different dishes was at approximately the same temperatures, there were no consistent differences between the percentages of germinating spores when exposed to diffuse sunlight and when in the dark.

Hydrogen-ion concentration.—A limited number of experiments were made with the standard buffer solutions of Clark (13) to determine the range of hydrogen-ion concentration in which teliospores will germinate. The solutions of phosphoric and thallic acid were diluted by adding one part of the standard solution to five parts of distilled water. The spores were sown on the surface of the liquid and tested in duplicate series at 18° C. The H-ion concentrations were checked first with a potentiometer and later by colorimetric tests. In the first germination tests made January 19 and repeated January 26, 1922, the spores responded in only a narrow range of H-ion concentration, pH 5.8–6.2. In April, spores from this same collection germinated in a wider range, pH 5.2–7.8. Another set of spores, collected in the fall of 1926 and tested in February, 1927, germinated in a pH range from 5.0 to 7.0.

The response of the spores in the first collection to a narrow range of H-ion concentration in January and a wider range in April is interesting.

It suggests that the maturity of the spores either may have been affected by, or caused by, changes in the reaction of the spore contents, resulting from the accumulation of products of oxidation. It is evident that some collections of teliospores will germinate in a fairly wide range of H-ion concentration, probably wide enough so that they would not be affected by fluctuations which might occur in the reaction of the teliospore sorus in nature.

Time required.—Different collections of teliospores differ considerably with regard to the time required for germination. Some of this difference may represent seasonal response. Deitel (19) was probably the first to suggest this. He found that teliospores of *P. graminis* from *Agropyron repens,* collected in March, required 30 hours to germinate; later, germination took place in $2\frac{3}{4}$ hours; in June $4\frac{1}{2}$ hours were required; and in July only a few spores germinated in 30 hours; in August no spores germinated, even within 36 hours. The writer has noted a similar seasonal response in the time required for germination, and, in addition, a tendency for spores from different sori on the same straw to require different lengths of time for germination. In 1924, spores from *Poa pratensis, Agropyron repens,* and *Agrostis alba,* collected in March, required 3 days to germinate, while in April spores from the same material germinated abundantly in 12 hours. Similar results were obtained again in 1925, with spores from *A. repens.* Collections of spore material have been tested which required 5 days to germinate. The minimum time for the germination of teliospores and the production of sporidia was approximately 6 hours. The average time was about 12 hours.

Most of the tests were made by scraping the spores off of the straw on to the surface of distilled water. In doing so, clumps of spores representing different sori from the same straw were often deposited intact on the water. In some dishes the time required for these individual clumps of spores to germinate varied from 12 to 24 hours. Eriksson (20, 21) believes that "Only the crop of spores maturing during the late autumn is able to germinate the following spring." This suggests the possibility that the difference in the response of spores in separate sori may be due in part to conditions at the time of the formation of the teliospores.

Both the seasonal response and the differences in the response of different material and of different sori may affect the period in which teliospores germinate in the spring. As a result, all of the teliospores in a given locality do not germinate in the first heavy rainfall. They germinate and produce sporidia during several rainy periods. This undoubtedly aids in synchronizing the time when teliospores germinate in the spring with the time when barberry leaves unfold.

Longevity of Mature Teliospores

Once teliospores have been shown to be viable in the spring, the following questions naturally arise: how long will these spores remain viable and what affects their longevity? It has been observed repeatedly that under natural conditions they usually are comparatively short-lived. Eriksson (20) noted that in the vicinity of Stockholm, Sweden, teliospores of *P. graminis* lost their viability in June. McAlpine (35) mentions the middle of October as the height of the season for germination in Australia, and states that, under favorable conditions, spores germinate until November, but germination never has been observed later.

Melhus *et al* (41) obtained plentiful germination in April and May, but spores left out of doors would not germinate in June or July. They observed that the cells of most of the spores became empty following heavy rains. The writer found that similar conditions existed at St. Paul, Minn., in 1923 and 1924, and was also able to distinguish clearly between germinated and non-germinated spores by the appearance of the spore contents.

Eriksson, Melhus and his co-workers, and Waterhouse have gone a step further and attempted to prolong the period of viability of the spores by drying and cooling them. Eriksson (20) was able to keep viable teliospores on *Avena sativa* and *Triticum vulgare* from May until September, simply by bringing them into a dry laboratory. Melhus *et al* (41) were able to keep them a few weeks longer by storing them in a cool dry place. Waterhouse (65) divided into two lots straw which contained viable teliospores in April, keeping one portion at ordinary laboratory temperature and the other in cold storage at a temperature a little above the freezing point. In the former, the percentage of germination began to decrease in June, while in the latter the spores germinated abundantly and were capable of infecting barberries until the end of July.

In April, 1925, the writer and R. U. Cotter[5] stored teliospores on *Poa pratensis* and *Agropyron repens* at different temperatures and relative humidities. Having a need for viable telial material to inoculate barberries in the varietal tests throughout the winter, a burlap sack was filled with the telial material on *Agropyron repens* and placed in cold storage at approximately 2° C.

In addition, bits of infected straw were sealed in 20 Mason jars which were kept at four different temperatures and adjusted to five different humidities. The results of germination tests with the material in these jars are given in table 2. It will be noted that material kept dry and at the

[5] Agent in the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

TABLE 2.—*The effect of temperature and moisture on the longevity of mature teliospores of Puccinia graminis secalis and Puccinia graminis poae, which germinated normally on April 25, 1925*

Stored April 25, 1925		Germination[a] tests, 1925									
		July 7				Nov. 11				Dec. 28			
Temperature, degrees C.	Relative humidity, per cent	*P. graminis poae*		*P. graminis secalis*		*P. graminis poae*		*P. graminis secalis*		*P. graminis poae*		*P. graminis secalis*	
		Trial		Trial		Trial		Trial		Trial		Trial	
		A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.
0–2	0	++	++	++	+	++	++	++	+	+	+	tr	tr
	20	++	++	++	++	++	++	tr	tr	+	tr	tr	tr
	40	++	++	tr	+	++	++	0	0	tr	0	tr	tr
	60	++	++	++	++	++	++	–[b]	–	tr	0	–	–
	80	++	++	tr	tr	tr	tr	++	tr	tr	tr	tr	tr
10–15	0	++	++	0	tr	0	0	+	+	0	0	0	0
	20	++	++	0	0	0	0	tr	0	0	0	0	0
	40	+	++	tr	tr	0	0	0	0	0	0	0	0
	60	tr	tr	0	0	0	0	0	0	0	0	0	0
	80	tr	tr	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0
	20	tr	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0	0	0	0	0	0
35	0	0	0	0	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0	0	0	0
	40	0	0	0	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0	0	0	0
	80	0	0	0	0	0	0	0	0	0	0	0	0

[a] 0 = no germination.
tr = few spores germinating.
+ = at least one clump of spores germinating abundantly.
++ = more than 10 per cent germination.

[b] Spores wet with acid.

lowest temperatures remained viable for the longest time. The tests with this material were not continued after December because most of the straw had, by that time, been used for the germination tests. However, Cotter continued his inoculations with the spores which had been kept in cold storage and was able to obtain infection until spring, when the old material was discarded for spores formed in the fall of 1926. This procedure has now become routine. Another set of spores which was placed in cold storage in the spring of 1926 was still being used to infect barberries in March, 1927.

It seems probable from these observations and experiments that most of the teliospores which mature in March or April will germinate in the spring; only a few will germinate in the summer because of high temperatures, but conditions may be favorable for germination again in the fall, and the remaining viable spores may germinate at that time.

Production, Liberation, Dissemination, and Germination of Sporidia

The conditions necessary for the production of sporidia appear to be essentially the same as those necessary for the germination of teliospores, except that teliospores will germinate under water, but sporidia are never produced under water. If a promycelium is produced under water, it will not bear sporidia unless the sterigmata grow out above the surface of the water. Promycelia from spores lying on the surface of the water usually are several times longer than those from spores on damp straw.

As pointed out by Buller (10), the sporidia usually are formed on the upper surface of the promycelium, where they sometimes germinate without leaving the sterigmata. A promycelium growing from a spore produced on a damp piece of straw almost always is shorter than one growing in water and is curved at right angles to the spore so that the sporidia are borne on sterigmata on the convex surface away from the straw. This production of the sporidia on the surface of the promycelium away from the spores is regarded by Buller (10) and Melhus *et al* (41) as an adaptation to facilitate the liberation of the spores.

Buller (10), in 1917, was the first to report that the sporidia of *P. graminis* normally are abjected forcibly from the sterigmata. He carefully observed the rate of the development of the spore at the end of the sterigmata and noted that the average time required for development from a tiny rudiment to a full grown spore was 20 minutes. It was estimated that a full-sized spore required about an hour of ripening before it was shot away. During this period of growth and ripening of the spore, the relative humidity of the air surrounding the spore must be comparatively high or the promycelium will collapse before the spore is fully formed and

matured. These observations clearly demonstrate that sporidia are liberated by a mechanism which assists in wind dissemination. Once the teliospores have matured, a moderately warm (10°–25° C.) 10-hour rain should be sufficient for their germination and the liberation of sporidia.

The mechanism for spore liberation makes it seem probable that sporidia of *P. graminis*, as well as those of other rusts (55), are disseminated by the wind. Aside from this, very little is known regarding the way these spores are distributed. Field observations indicate that teliospores usually can be found within a quarter of a mile of infected barberries. Bushes in city plantings flanked by clipped lawns seldom have more than a half-dozen cluster cups on them.

The writer has examined hundreds of barberry plantings and has found teliospores within a hundred yards of the bushes in all cases in which the barberries were infected heavily enough to be a factor in the development of a destructive epidemic. Owing to their boat-like shape, sporidia have an enormous surface as compared with their mass. Therefore they probably are carried long distances by the wind, and there probably is a general dissemination of sporidia. But this general dissemination is apparently too sparing to cause a heavy infection on barberries.

The optimum temperature for the germination of sporidia (41) is between 15° and 20° C. They oftentimes germinate at 18° and 20° C. while still attached to the sterigmata; they will also germinate on solid media, apparently not requiring actual contact with drops of water.

INFECTION OF BARBERRIES AND THE LIBERATION, DISSEMINATION, AND GERMINATION OF AECIOSPORES

The importance of barberries as a source of inoculum depends on the physiologic form of stem rust present, the severity of infection, and the number of spores produced. These in turn depend on a number of factors: the species, abundance, and proximity of susceptible grasses and cereal hosts, the severity and time of infection in the previous year, and weather conditions. Emphasis will be placed here on the relation of weather conditions to the geographic distribution of barberry rust and to the seasonal fluctuation in severity of infection.

Geographic Distribution of Barberry Rust

The maturation and germination of teliospores in spring are important factors determining the distribution and severity of barberry rust. Field observations[6] over a period of eight years indicate that, in the Mississippi Valley, barberry rust is most abundant in the Northern States, sparing in

[6] Unpublished papers of Stakman *et al.*

Missouri and Kansas, and practically absent in Texas and Oklahoma. This distribution appears to be due largely to the viability of teliospores.

Stakman, Kirby, and Thiel (57) have shown this by field experiments. They inoculated barberries in the south with three types of inoculum: teliospores which were formed and matured in the south, spores which were formed in the south and kept over summer and winter in the north, and spores developed in the north. It was found that barberries would not become infected in the Southern States when inoculated with teliospores matured in the south. On the other hand, when they were inoculated with spores kept through the summer and fall in the north, excellent infection was obtained whether the spores were formed in the north or the south. They state, "Numerous germination tests were made and the results of the germination tests correlated with the facts observed regarding infection. Evidently, therefore, the reason why barberries do not become infected in the south is not because conditions are unfavorable for infection, but because practically no teliospores are viable in the spring."

The reason for this lack of viability has not been definitely ascertained. There are two possibilities. The teliospores either germinate during the winter or they are killed by the high temperatures which prevail during the hot summers in the south. Controlled experiments, which have already been discussed, tend to substantiate the latter hypothesis. In this connection it is well to recall that in Texas, Oklahoma, southern Kansas, and southern Missouri, teliospores are formed in May and June, and therefore must endure the hot months of July, August, and September. In the spring wheat area, they are formed mostly in July and August, and it is not necessary for them to withstand so long a period of hot weather.

Severity of Barberry Infection

Temperature.—Assuming the presence of viable teliospores near the barberry, the two most important factors determining the abundance of infection seem to be temperature and moisture. Light plays only a minor rôle. Temperature appears to be a factor in determining the season of the year when barberries become rusted and the date of primary infection, while rainfall often determines the amount of infection in a given locality.

As mentioned previously, the temperature range for the germination of teliospores and the production of sporidia was determined by earlier workers to be between 10° and 25° C., with an optimum at 20° C. It was also determined that the germination of sporidia occurred principally between 15° and 20° C. To supplement these observations, Melander[7] (40) and the

[7] These experiments were made jointly by the writer and L. W. Melander, who is Associate Pathologist in the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in charge of barberry eradication in Minnesota.

TABLE 3.—*The effect of temperature during incubation upon the infection of Berberis vulgaris with Puccinia graminis secalis*

	Temperature, degrees C.	No. plants used	No. leaves		No. petioles infected	No. shoots		Degree of infection
			inoculated	infected		inoculated	infected	
First trial June 1, 1923	17–18	8	163	105	42	22	20	Heavy
	22–23	8	154	52	10	7	5	Light
	26–28	8	130	0	1	12	0	One small pustule
	31–33	8	142	1	0	13	0	One small pustule
Second trial June 20, 1923	17–18	4	76	25	3	0	0	Heavy
	22–23	4	80	46	6	0	0	Light
	26–28	4	95	0	0	0	0	———
	31–33	4	106	0	0	0	0	———

writer attempted to determine the effect of temperature, under controlled conditions, on the extent and severity of barberry infection. Straws bearing viable teliospores were tied to barberries, which were then sprayed with water and immediately placed at the bottom of sealed constant temperature tanks. To facilitate infection, the air in these tanks was kept at approximately 100 per cent relative humidity for 72 hours. The degree of infection was noted 10 days after inoculation. By that time aecia already had developed on many of the leaves. As indicated in table 3, the greatest amount of infection occurred at 17–18° C. It was slight at 22–23° C., and there was little or none at 26° C. This experiment indicates that the combined process of teliospore germination, sporidia formation, liberation, and germination, the infection of barberry leaves, and the development of aecial cups are retarded or prevented by temperatures higher than 22° C. This comparatively low optimum for infection is probably one of the reasons for the scarcity of barberry infection in the summer.

In spite of the fact that fresh aecia usually are scarce in midsummer, in certain seasons barberries may become quite heavily rusted in September. This was especially noticeable in Minnesota in the fall of 1921. This fall infection may have been due to germination of newly formed teliospores, but when it is remembered that the summer of 1921 was exceptionally dry, and that dry teliospores may remain viable several months after they mature, another explanation for the fall infection suggests itself. The teliospores may have remained viable throughout the summer, but the rains were not cool enough for germination and infection until September. Thus the low optimum temperature for infection apparently is expressed in the field by abundant spring infection, scarcity of barberry rust in the summer, and an occasional renewal of infection in the fall. The comparative abundance of barberry rust early in the spring is probably also due partly to the fact that only young barberry leaves are highly susceptible.

Because of its earliness, this primary infection is usually the most important one in the furnishing of inoculum for the development of widespread epidemics. The dates of first infection on barberries have been recorded accurately in Minnesota for 1921–26.[8] The comparative severity of state-wide infection also has been noted. These observations are summarized in table 4. The outstanding features of these six years' observations are the early infection in 1921, the late infection in 1924, and the comparatively light infection in 1926.

An attempt has been made to determine if unusual weather conditions were associated with peculiarities in the infection of barberries in these

[8] Unpublished papers of Stakman *et al.*

TABLE 4.—*The dates of first appearance of pycnia and the comparative state-wide severity of rust on barberries in Minnesota, 1921 to 1926 inclusive*

Year	Date of first infection	Stage of development	County	Observer	Comparative state-wide severity of rust
1921	April 25	Pycnia	Jackson	L. W. Melander	Heavy
1922	May 4	do	Rice	do	do
1923	4	do	do	J. J. Christensen	Moderate
1924	12	do	Goodhue	L. W. Melander	do
1925	1	do	Olmstead	do	do
1926	1	do	do	do	Light to moderate

years. The departures from the mean monthly temperatures in March, April and May of these years are given in table 5.

These departures suggest that two factors operated to produce the early infection in 1921: first, the month of April was much warmer than usual; and second, rains occurred from the 6th to the 9th which were heavy enough to delay farm work. Since April, 1925, was also warmer than normal, infection might be expected in that year earlier than May first, but during April, 1925, there was a short cold spell near the end of the month when temperatures in general fell considerably below the freezing point. This probably explains the delayed infection in that year.

A similar cold spell which occurred early in May, 1924, seems to be correlated with the exceptionally late infection in that year. "The month was abnormally cold. In all the 34 years' record there was but one made, that of 1907, which was colder. . . . A warm period of two or three days' duration began on the 15th and throughout the remainder of the month it was consistently cold, especially during the first week when freezing was

TABLE 5.—*The departure from the normal temperature*[a] *for March, April, and May, in Minnesota, 1921 to 1926 inclusive*

Year	March	April	May
1921	+2.7	+3.5	+2.1
1922	+2.1	−0.3	+5.2
1923	−8.4	−2.6	+1.0
1924	+1.2	−1.8	−7.3
1925	+4.3	+5.7	−2.2
1926	−2.8	−1.9	+4.4

[a] The 36-year averages given in Climatological Data for 1926 were used as normal; they were March 26.3°, April 43.3°, and May 55.0° F.

the rule.''[9] There appears to be no correlation between the temperature in March and the earliness of barberry infection.

Rainfall.—The light infection which occurred in Minnesota in 1926 seems to be correlated with the exceptional drought during April, when there were only three days with measurable precipitation in the month. The last two weeks in April is usually the period when barberry leaves begin to develop in Minnesota. As shown in figure 2, there was a general

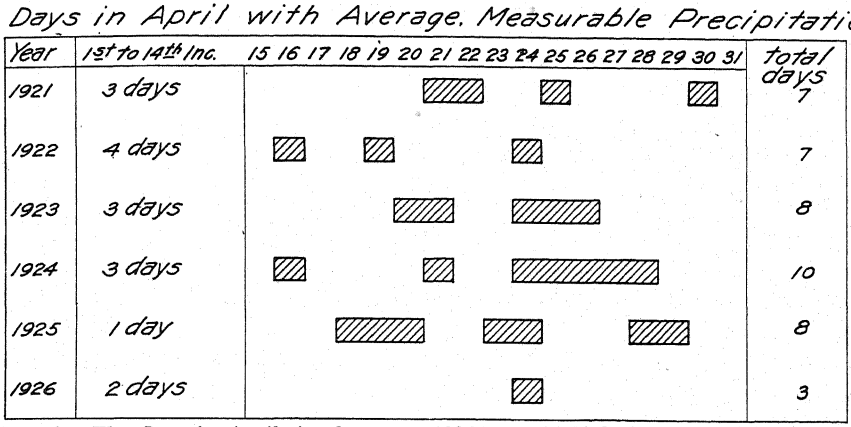


FIG. 2. The days in April, in the years 1921 to 1926 inclusive, on which an average measurable precipitation occurred throughout Minnesota.

measurable rainfall on only one day during this period in 1926. The month was ''by far the driest April in all the 36 years' record.''

In May, 1926, precipitation also was decidedly below normal, although the drought was not so pronounced as in the previous month. Since experiments with teliospores indicate that an approximately saturated atmosphere is necessary for about 12 hours previous to barberry infection, it seems reasonable to account for the ''light to moderate'' infection in Minnesota in 1926 (Table 4) on the basis of this April and May drought.

Local droughts also have been observed to affect barberry infection. Melhus *et al* (41) point out that the distribution of midsummer infection in northern Iowa appeared to be markedly influenced by the distribution of rainfall. Similarly, the combined observations of Mr. A. F. Thiel and Mr. H. A. Rodenhiser[10] in the spring of 1926, indicate that barberry infection in north-central Kansas was delayed by drought for a period of from two weeks to a month.

[9] See the Minnesota section of Climatological Data for May, 1924.

[10] Unpublished papers of Stakman *et al.*

Light.—In 1923 controlled experiments were made by Melander (40) and the writer, to learn something of the relation of light intensity to the infection of barberries and subsequent development of aecia. The telial material used for inoculum was on straw of *Agropyron repens,* and the rust was probably *Puccinia graminis secalis.* The experiments were made in a room where the only source of light was from several 1,000-watt nitrogen electric light bulbs spaced about 1½ feet apart on the ceiling and 6 feet above the plants.[11] The plants were set under the lights so as to allow as uniform a distribution of light as possible. Two intensities of light were used. The intense light measured approximately 270 foot candles, and the diffused light—under two layers of cheesecloth—measured about 200 foot candles. Temperature was kept fairly constant by a thermostat and fan. The plants were kept in approximately 100 per cent relative humidity for 72 hours in three different ways: exposed to constant intense light, alternately exposed to constant intense light and total darkness for 12-hour periods, and kept in total darkness. Twelve plants were incubated in each of these three ways. At the end of the incubation period, the three series of twelve each were divided into three small sets of four plants. These small sets were then placed in constant intense light, intense light alternating with darkness, and alternating diffused light and darkness. This was done to determine the effect of different light conditions, presumably after penetration had occurred. Table 6 summarizes the percentages of infection after 10 days of incubation. All of the plants were infected. Different amounts of light during the period of incubation apparently had no effect on the percentage of leaves infected, but the number of infected petioles was markedly greater on the plants kept in total darkness than on those exposed to constant intense light. After incubation, more intense light apparently increased the severity of infection both on leaves and petioles. The increased infection on petioles in the darkness was probably due directly or indirectly to etiolation. The higher degree of infection on plants exposed to intense light after incubation may have been correlated with metabolism in the host leaves. Probably neither effect was pronounced enough to influence the amount of infection in the field.

Liberation of Aeciospores

Although the forcible discharge of aeciospores in the rusts was described by Zalewski (67), in 1883, it was not until 1924 that Buller (10) described the discharge of aeciospores of *Puccinia graminis.* In still air they are

[11] The use of this room was made possible through the courtesy of Dr. R. B. Harvey, Associate Professor of Plant Physiology and Botany, University of Minnesota.

TABLE 6.—*The effect of different light intensities on the severity of Puccinia graminis secalis on Berberis vulgaris*

ht con- tions ıring ıbation moist mbers	Light conditions after removal from moist chambers															
	Alternating intense light and darkness						Alternating diffuse light and darkness						Constant intense light					
	Serial no. of individual plants	Total no. leaves inoculated	Infected leaves		Infected petioles		Serial no. of individual plants	Total no. leaves inoculated	Infected leaves		Infected petioles		Serial no. of individual plants	Total no. leaves inoculated	Infected leaves		Infected petioles	
			No.	Per cent	No.	Per cent			No.	Per cent	No.	Per cent			No.	Per cent	No.	Per cent
·nating	31	26	14	53.8	11	42.3	33	48	20	41.7	12	25.0	26	25	17	68.0	12	48.0
ht and	29	39	23	59.0	10	25.6	35	27	15	55.6	3	11.1	25	20	13	65.0	8	40.0
rkness	32	61	42	68.8	7	11.5	36	75	35	46.7	5	6.7	28	39	20	51.3	5	12.8
	30	6	1	16.7	0	00.0	34	70	4	5.7	1	1.4	27	34	11	32.4	4	11.8
Totals	4	132	80	60.6	28	21.2	4	220	74	33.6	21	9.5	4	118	61	51.7	29	24.5
	19	14	14	100.0	9	64.3	24	21	18	85.7	12	57.1	14	13	11	84.6	10	76.9
tant	18	51	22	43.1	32	62.7	22	16	6	37.5	3	18.8	15	39	29	74.4	15	38.5
rkness	20	15	6	40.0	8	53.3	21	14	11	78.6	2	14.3	16	30	11	36.7	5	16.7
	17	53	17	32.1	18	34.0	23	31	7	22.6	4	12.9	13	28	6	21.4	2	7.1
Totals	4	133	59	44.4	67	50.4	4	82	42	51.2	21	25.6	4	110	57	51.8	32	29.9
	11	42	39	92.8	18	42.9	10	36	17	47.2	13	36.1	8	19	8	42.1	5	26.3
tant	6	15	10	66.7	5	33.3	7	70	54	77.1	15	21.4	1	34	20	58.8	8	23.5
;ht	9	37	23	62.2	3	8.1	3	22	1	4.5	2	9.1	5	22	16	72.7	3	13.6
	2	65	36	55.4	2	3.1	12	18	2	11.1	1	5.6	4	55	23	41.8	1	1.8
Totals	4	159	108	68.6	28	17.6	4	146	74	50.7	31	21.2	4	130	67	51.5	17	13.1

shot, on the average, 4–5 mm. from the aecial cup (10 and 15). They are discharged singly or in clumps and only in approximately saturated atmosphere. The maximum rate of discharge may be reached in an hour and a half after the cups are moistened (15). It is evident that the mechanism for the liberation of aeciospores facilitates wind dispersal.

Dissemination of Aeciospores

Earlier workers apparently did not distinguish between dissemination of aeciospores and the secondary spread of rust from urediniospores when tracing the development of local epidemics near barberries. Epidemics near barberries have been noted for nearly 200 years. During the latter half of the ninteenth century, aeciospores and the rust produced by them were assumed to be the source of infection for the whole neighborhood. Since the barberry eradication campaign has been in progress in the United States, numerous cases have been reported in which the rust had undoubtedly spread several miles. However, very few attempts have been made to determine the distance to which aeciospores spread from barberries.

The writer often has observed primary infection from barberries, but knows of only one case in which a large, heavily infected planting was found early enough in the season to distinguish between the primary infection due to aeciospores and the infection due to the spread of urediniospores. This was at Northfield, Minnesota. The planting was especially favorable for study: first, because it consisted of 175 large, severely rusted bushes, most of them 6 feet or more in height; second, because it was located on a deserted farmstead which was slightly higher than the nearby fields and was almost free of buildings and trees; third, because susceptible cereals and grasses were abundant in adjacent fields. Dr. J. J. Christensen[12] made some interesting observations on the spread of aeciospores from these bushes in the spring of 1922. The following data are taken from his summary. On May 18, when the bushes were first observed, the aecial cups were just beginning to open. A few days later there was an average of eight aecial clusters on each infected leaf. It was estimated that each bush produced at least 64,512,000,000 aeciospores, making a total of 11,289,600,000,000 spores. This estimate was very conservative, since it purposely did not take into consideration the elongation of aecial chains.

In order to determine how many spores were being deposited on surrounding fields, vaseline-coated glass slides, 1 inch by 3 inches, were exposed vertically about 3 feet from the ground at varying distances from the bushes. These slides, in addition to determining the number of spores, and the distances to which they were disseminated, made it possible to dis-

[12] Unpublished papers of Stakman *et al.*

tinguish the infection due to aeciospores from that due to newly formed urediniospores, by establishing the date when urediniospores were first disseminated. On May 24 and 25, when the slides were first exposed, the spores were being liberated at a tremendous rate. Several slides were exposed for 20 hours; one 3 feet from the barberry hedge caught approximately 160,000 spores; on another 6 feet away there were 33,000 spores; while on one 23 yards from the bushes there were 210 spores. A few days later, more than 3,000 aeciospores were caught on slides which were exposed for a week, at a distance of five-eighths of a mile from the bushes. A few aeciospores were caught on a slide exposed for 48 hours one mile from the bushes.

Because no urediniospores were found on the slides until June 2, it was assumed that all rust found on grains and grasses prior to and including June 6 was due to aeciospores only. A survey, made on that date by Dr. Christensen, Mr. G. D. George, and the writer to determine the extent of the infection, indicated that rust had spread more than a mile and a half from the barberries. It was heavy on volunteer rye and *Agropyron repens* for at least half a mile from the barberries, gradually decreasing as the distance from the bushes increased, until finally no trace of rust could be found on susceptible hosts. The infected area extended a mile west and north of the bushes and a mile and a half east, but only a few rods south. This peculiar shape of the rusted area was apparently due to the dissemination of spores by southeast and southwest winds.

The observable dissemination of aeciospores from the barberries was clearly limited to a two-mile radius from the bushes. Since these bushes were more heavily rusted and more favorably situated for disseminating spores than the average barberry planting, it seems probable that in the majority of cases observable primary infection from aeciospores is limited to the immediate vicinity of the bushes. Millions of spores were caught on the slides near the bushes, and there is evidence (56) that these spores may be lifted by the wind to elevations of several thousand feet. It is probable that aeciospores are disseminated many miles by the wind, but the infection centers which the individual spores cause are apparently too far apart to be found by the ordinary field survey.

Germination of Aeciospores

In 1865, De Bary (16) reported that aeciospores germinated very poorly. Since that time most of the investigators (15, 22, 35, 48 and 53) who have attempted to germinate aeciospores have experienced some difficulty, and concur in the opinion that the germination of the aeciospores of *Puccinia graminis* is remarkably capricious. Craigie (15), who has made probably the most extensive experiments, was not able to get uniform results. He

found that freshly discharged spores germinated in an hour and a half to two hours. The highest average germination took place in distilled water between 5° and 18° C. Very few spores germinated above 22° C. A few spores germinated after having been kept in the laboratory two weeks.

OVERWINTERING OF THE UREDINIAL STAGE

After the demonstration of heteroecism in the rusts by De Bary (16), certain investigators (1, 8, 42, 48, and 49) were inclined to believe the aecial stage necessary for the reinvigoration of the pathogene. Freeman and Johnson (24) proved that the sexual stage was not essential by culturing the uredinial stage through 52 transfers without any apparent loss of vigor. They also summarized the earlier work on the overwintering of the uredinial stage. The severe epidemic in 1904 first focused attention on the question of whether urediniospores may continue to propagate themselves in the Mississippi Valley.

Bolley and Pritchard (8) believed that field evidence did not preclude the possibility of overwintering occurring on the seed or as mycelium or spores on fall-sown grain or perennial grass hosts.

Seed Transmission of Stem Rust

Pritchard (51 and 52) found uredinia and telia of *P. graminis* on the inside of wheat kernels, and cites Smith as observing telia in oat seed in 1884. He believed the fungus overwintered in seed wheat, and concluded that "the mycelium from the pericarp penetrates various parts of the seedling, invading both the intercellular spaces and the cells. It soon passes into the spaces between the leaf sheaths, where it grows rapidly and attacks the tissues at various points. This peculiar mode of travel and attack may explain the failure of Eriksson and Ward to trace the fungous filaments beyond the immediate confines of the first appearing pustules, for these areas of infection may not spread while being carried up by the growth of the plant." He suggested that experiments should be made in which plants from infected seed be compared with plants from seed treated with Jensen's hot water treatment.

Hungerford (27) made careful experiments of this kind and was unable to demonstrate any difference in the amount of rust on plants from infected seed, inoculated seed, treated seed, and clean seed. His conclusions are as follows: "The results of experimental work here reported indicate that stem rust is not transmitted from one wheat crop to the next by means of seed grain. Further, in the writer's judgment, the occurrence of stem rust sori in the pericarp of the caryopsis of grains and grasses has no especial

significance, but the infection spreads to these tissues just as it does from an infection point in any of the vegative parts of the plant."

This method of overwintering does not seem likely and will not be considered further.

Overwintering in the Field

It has been known for nearly a quarter of a century that viable urediniospores of stem rust may persist throughout the winter in the Mississippi Valley. But there is a preponderance of evidence that they do not cause widespread infection on cereals in the spring in the Northern States.

During the winter of 1904–1905, Bolley and Pritchard (8) surveyed fields throughout the Mississippi Valley and made collections of fresh viable spores of *P. graminis*, at Dallas, Texas, December 25; and in December and January in Oklahoma, Kansas, Missouri, Mississippi, Illlinois, Wisconsin, Minnesota and North Dakota. He states that "Quantities of them (viable urediniospores) were being produced on winter wheat at Riverside, Illinois, . . . and a quantity of viable spores was taken from quack grass and wild barley frozen in ice at Fargo in March, 1905." In the winter of 1906–1907, Freeman and Johnson (24) germinated urediniospores of *P. graminis* from *Hordeum jubatum, Agropyron repens,* and *A. tenerum* throughout the winter and early spring months. Some of these spores, collected in the vicinity of St. Paul, Minnesota, germinated as late as April 15. But they never were able to obtain viable spores late enough in the spring to cause infection in the field. Similar results were obtained by Dickson and Johnson (18).

For the past eight years, 1918–1926, extensive studies have been made throughout the Mississippi Valley to determine the northern limits of overwintering in the uredinial stage.[13] These studies consist of germination tests, tests of the longevity of mycelium in wheat seedlings, and observations of primary infection in the spring. In general they indicate that, north of Oklahoma, primary infection of cereals as a result of local overwintering in the uredinial stage, occurs so rarely that it is probably of no practical importance. There is circumstantial evidence of overwintering in certain years in northern Texas and Oklahoma. It undoubtedly occurs in most years in a small percentage of fields of wheat and oats in the region around San Antonio, Texas. Although it is known (61) that *P. graminis phleipratensis* may overwinter in the Northern States, no evidence has been presented that the stem rusts of cereals overwinter on grasses and spread to grain in the spring.

It is not within the scope of this paper to present evidence for the geographic distribution of uredinial overwintering. We are interested rather

[13] Unpublished papers of Stakman *et al.*

in determining the conditions which establish this distribution. It is almost axiomatic that climatic conditions are responsible for confining overwintering to the south, but the specific conditions principally concerned are certainly not self-evident. The zone of demarcation between a region where stem rust overwinters frequently and the nearest region where it does not overwinter would seem to be the most favorable place to study this problem in the field.

It has already been mentioned that, with few exceptions, the northern limits of overwintering appear to be in northern Texas or Oklahoma. In this region an extensive survey was made in the winter of 1923–1924. Wallace Butler observed conditions around San Antonio, and Frank Frolik studied conditions in northern Texas and Oklahoma.[14] Stem rust of wheat overwintered in Butler's artificially inoculated plots at San Antonio and Austin and in a naturally infected field at Cliff, which is about 20 miles from San Antonio. Stem rust of oats also overwintered in the experimental plots of San Antonio and in a naturally infected field at Uvalde. Frolik examined 512 fields of wheat but was unable to find any evidence of overwintering. He obtained circumstantial evidence of abundant overwintering on volunteer oats in the vicinity of Elgin and Taylor, Texas. Farther north, at Temple, Waco, and West, the rust seemed to have overwintered in an occasional field of volunteer oats, but there was no evidence of overwintering on oats in the vicinity of Dallas and farther north. It was evident that conditions at San Antonio favored overwintering, and that the district around Dallas was the farthest south in which conditions were distinctly unfavorable for overwintering.

Butler charted the daily weather conditions at San Antonio, and Frolik charted and compared those at Dallas and San Antonio. Figure 3, A and B, gives the daily maximum and minimum temperature and the amount of precipitation at these two places for the period from June, 1923, to May, 1924, inclusive. There were two elements of difference between the weather at San Antonio and that at Dallas which seemed to have a bearing on the problem. In the first place, periods of alternate freezing and thawing occurred only three times during the winter at San Antonio, while at Dallas they occurred nine times. Secondly, there were no periods during which the mean temperature was over 65° F., accompanied by sufficient rainfall for infection, in the latter half of December, January, February, or early March at Dallas, while four such periods occurred at San Antonio. There was sufficient rainfall for spore germination on several occasions at Dallas in January and February, but as no fresh pustules appeared in February

[14] Unpublished papers of Stakman *et al.*

and March it was assumed that the temperature was too low for infection during these months.

These observations suggest that alternate freezing and thawing are critical conditions for stem rust. Under these conditions uredinospores germinate prematurely and the host leaves bearing the old pustules are killed. North of Oklahoma, artificial inoculation as a rule will not cause infection after September. In the central Mississippi Valley, periods of alternate freezing and thawing occur frequently throughout the winter and spring. In the Northern States, repeated germination tests have shown that urediniospores may remain viable throughout the winter but are eliminated in the spring, during periods of alternate freezing and thawing.[15]

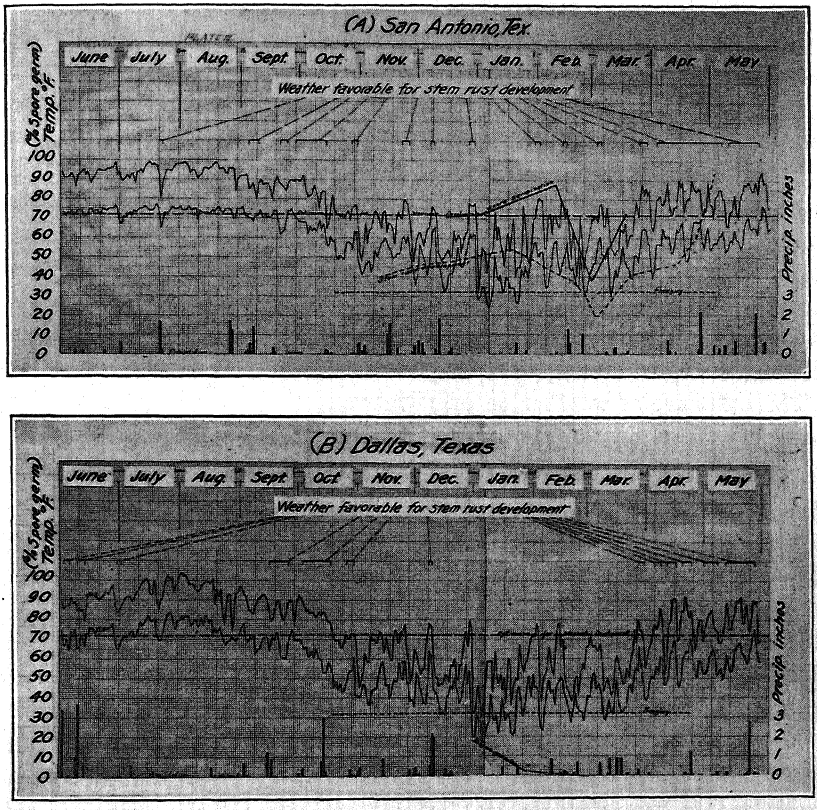


FIG. 3. Temperature range and amount of precipitation at (A) San Antonio, Tex., and (B) Dallas, Tex., from June 1, 1923, to May 30, 1924. Brackets indicate the periods favorable for stem rust development, *i.e.*, periods during which the mean temperature was between 65° and 78° F., accompanied by abundant precipitation.

[15] Unpublished papers of Stakman *et al.*

Weather conditions during the previous growing season also may affect overwintering by determining the amount of inoculum present in the fall, or the condition of the host. There is very little evidence of overwintering north of Oklahoma, in spite of the fact that inoculum usually is plentiful in the fall. Therefore conditions during the previous growing season are probably not important in the Northern States. In the Southern States inoculum is not always present in the fall. This is especially true in central and southern Texas. For several years Butler has shown by inoculation experiments that stem rust will overwinter at San Antonio when inoculum is present in the fall.

On the other hand, field observations indicate that the rust overwinters in only one field in a hundred in this region. It is difficult for uredinial infection to persist on grass hosts through the latter part of June, July, August, and September, during which period no grain is present because of the early harvest and late fall sowing. These are hot months in Texas, and there is usually little precipitation until October. The grasses dry up early in the summer, except for a few green patches along the stream beds. There is some evidence that local oversummering occurs in situations of this kind, but it has never been clearly demonstrated that stem rust spreads from susceptible grasses to grain in the fall in Texas. It is not clear whether fall infection occurs as a result of local oversummering or wind-blown spores, but it is certain that, in the San Antonio area, oversummering is more difficult for the rust than overwintering.

The influence of weather on overwintering through its effect on the host plant as well as on the pathogene is exemplified by the conditions which brought about the predominance of stem rust on oats over that on wheat in northern Texas and Oklahoma in the season of 1925–1926. In the opinion of the writer this situation was due to a peculiar chain of circumstances beginning in the winter of 1924–1925, which were unusually favorable for the development and persistence of volunteer oats. In the spring of 1925, because of a drought, most of the oat crop was not worth cutting. It was pastured off, and the cattle trampled the seed into the soil, where it remained until fall. Early fall rains favored the germination of this seed.

With the exception of two cold weeks in October, the winter of 1925–1926 was extremely mild in northern Texas and Oklahoma. Volunteer oats flourished and was the most vigorous crop in that region. Uniform stands were obtained and most of the fields were allowed to grow to maturity. They were infected in the fall and apparently furnished ideal conditions for the overwintering and early spring development of stem rust. Incidentally, a heavily rusted field of volunteer oats was found in June, 1926, near Enid,

in northern Oklahoma, which strongly suggested overwintering. The wheat situation, on the other hand, was in direct contrast with this condition. Volunteer wheat was scarce in Oklahoma, probably because of loss of viability of the seed during the hot summer months, and winter wheat was delayed by cold weather in October and apparently was not infected in the fall.

Thus it appears that stem rust of oats predominated over that on wheat in the spring of 1926, because conditions which prevailed in northern Texas and Oklahoma during the winter of 1924–1925, the spring and summer of 1925, and the winter of 1925–1926, continued to favor the development of volunteer oats but were not especially favorable for volunteer wheat.

DISSEMINATION OF UREDINIOSPORES

All of the spore forms of stem rust except the teliospore are adapted to wind dissemination. The sporidia and aeciospores are shot away from the host, while the urediniospores accumulate in a powdery mass in the sori until they are picked up or shaken off by the wind. Observations on the spread of rust from centers of primary infection in wheat fields, and on the spread of rust from infected grain near barberries, have given direct evidence that urediniospores may be disseminated several miles. There is strong circumstantial evidence that viable urediniospores may be transported several hundred miles. Local dissemination of spores that actually can be traced from their source will be considered first.

Local Dissemination

Field observations indicate that a considerable quantity of uredinial inoculum must accumulate before stem rust migrates in perceptible amounts from one field to another. This is illustrated by the spread of rust from individual pustules in the general primary infection in the spring wheat area, from spot infections in Oklahoma, Missouri, and Illinois, and from overwintering centers in southern Texas.

For the past six years, 1920 to 1926, a general primary infection has appeared in June almost simultaneously in practically every wheat field over a large part of the spring wheat area. In most fields this infection consists of single pustules on less than 1 per cent of the plants. The development of rust centers from these single pustules has been studied on several occasions, and small areas developed from them have been repeatedly found in all stages of development. On the average, these areas develop as follows:

First generation—single pustules on leaves or culms of plants, from 3 feet to a rod apart in the field.

Second generation—several fresh pustules near the old pustule, usually on the same plant, with possibly one or two pustules on adjacent plants.

Third generation—several culms with 1–5 per cent infection, each plant within a 2-foot radius having one or several pustules, usually on the side of the culm toward the old infection.

Fourth generation—heavily rusted areas coalescing but usually still discernible, average infection 1–5 per cent throughout the field.

Fifth generation and sixth generation—infection practically uniform throughout the field.

Since infection becomes more or less uniform in the spring wheat area after the fourth generation, it is impossible to determine in that region the size that isolated centers of infection might attain. However, in Texas, Oklahoma, Kansas, Missouri, and Illinois, isolated infection centers often develop. These sometimes become rather large without materially influencing the amount of infection outside of the immediate vicinity of the rust center. A few examples will serve to illustrate the nature of this infection.

Perhaps the most striking cases were found by Butler[16] in 1922 in Kansas and Missouri. In a field of wheat at Parsons, Kans., June 4, a spot about 20 feet across was discovered in which infection ranged from 1 per cent to 25 per cent on practically all plants. Twenty feet from the edge of this area no rust could be found, nor was any rust found in a search through a half-mile of the field. There were no peculiarities in the topography of the field which would explain the limited size of the rust spot. After examining plants for some time in a nearby field, a second spot was discovered, slightly larger, with about 25 per cent severity in the center and light infection at the edges. A careful search failed to reveal any stem rust at other points. In the next three days similar spots were found at Clinton, Newburg, and Springfield, Mo. By June 8, when the spot was found at Springfield, traces of stem rust were beginning to appear in most wheat fields in northern Missouri, but the rust had not developed further than the second generation outside these rust spots. The severity and prevalence of the rust was determined in a field containing a typical spot near Springfield. In this field there was about 25 per cent severity inside an area 10 feet in diameter; 25 feet from this area, an average of four pustules on 40 per cent of the plants; and in the most remote part of the field, from one to three pustules on 12 per cent of the plants.

In 1924, Frank Frolik and the writer found rust areas 10–15 feet in diameter near Dallas and Denton, Tex. In 1925, William Walker and the

[16] Unpublished papers of Stakman *et al.*

writer found numerous spots 2–6 feet in diameter on oats in northern Texas. The same year spots 2–15 feet in diameter were found by Frolik in Gallatin and Saline Counties in Illinois.

In all these cases the limits of the infected areas were quite distinct, indicating that stem rust usually spreads slowly for the first four or five generations, until a heavily rusted area is developed 5–10 feet in diameter, which usually contains sufficient inoculum to cause a noticeable infection over several acres. When rust has become plentiful in an entire field, it may spread to surrounding fields, at least within a radius of from one to several miles. Evidence of this has been found in Texas and Oklahoma and near barberries in the Northern States.

On June 2, 1922, a field of wheat was found near Denison, Tex., in which stem rust infection averaged 50 per cent on all plants. Here the rust had apparently already spread to four other fields within a mile of this field, since there was between one-half and one-fourth as much infection in these fields. The infection tapered off as the distance from the heavily rusted field increased.

A similar center of infection was found in a field of oats near San Antonio, Tex., in 1923. On May 1, there was a 50 per cent infection on most plants over a considerable portion of this field. Another field a hundred rods away contained from a mere trace to 5 per cent. A careful search was made in 20 fields of oats which were located from 2 to 20 miles from this field. Two pustules were found in one field and none in the others.

In 1924, stem rust overwintered in a mixed field of Kanred and Arnautka (Nicaraugua) wheat near Cliff, Tex. Cliff is less than 25 miles from San Antonio. On March 27 there was a trace of rust on approximately 1 per cent of the plants. On April 8 rust had not yet spread to two fields which were located within a mile of this field. By May 10 the rust in the former field had reached a severity of 10–40 per cent on most plants. In a field 40 rods away the plants nearest the heavily rusted field had from 1 pustule to 10 per cent of rust on them; 60 rods farther only traces of rust were present. On May 16 the first field was in the hard dough stage, and an average of 75 per cent rust had developed in it. On this date a field of the same variety a mile east contained no more than a trace or 1 per cent rust. At harvest time, June 1, the wheat in the field in which stem rust had overwintered was lodged, because of the extremely heavy rust infection; a 40 per cent infection had developed on the near side of the field 40 rods away, the infection tapering off to 3 per cent on the opposite side of the field; and in a field of the same variety, one mile east, the rust had increased

to between a trace and 2 per cent. Spot infections were found in several wheat fields along the road from Cliff to San Antonio.

An excellent example of rust spreading for several miles from a heavily infected field to surrounding territory was observed by the writer near Enid, Oklahoma, June 9, 1924. Numerous spots of rust in the third generation were found in a 30-acre field 9 miles south of Enid. This field proved to be in the center of a rusted area. There were traces of rust in fields as far as 3 miles south of the heavily rusted field and 6 miles north, but outside of this area no rust could be found. Previous to June 9, two weeks had been spent searching in vain for stem rust in southern Kansas and northern Oklahoma.

After discovering the rusted area, a careful survey failed to reveal any stem rust along the Meridian Highway between Enid, Okla., and Wellington, Kans., a distance of 40 miles. Butler, who drove north from Texas on this same road a few days earlier, found rust in southern Oklahoma, at Duncan, but could not find it in 21 fields between Duncan and El Reno. These observations leave little room for doubt that the rust had spread at least 3 miles south and 6 miles north from the one heavily rusted field near Enid.

Some of the best evidence of local epidemics due to wind-blown urediniospores from heavily rusted fields may be found in the secondary spread of rust from barberries. The epidemic near the barberries at Northfield, Minn., may be used again as an illustration. It will be recalled that on June 6 uredinial infection, due to the dissemination of aeciospores, had spread 1½ miles from the barberries and covered an area of about 2½ square miles. On June 12, urediniospores from this area, reinforced by newly formed aeciospores, had spread rust over an area covering about 6 square miles. By June 18 the heavily rusted area of rye and *Agropyron repens* included 12 square miles, and on July 10 it included approximately 40 square miles. By that time the epidemic from the planting at Northfield had coalesced with rust spreading from six smaller plantings which were found nearby.

This positive evidence of the spread of rust over 40 square miles in a few weeks raises the question of how far rust may spread from such an area.

Regional Dissemination

The diffused nature of the primary infection in the spring wheat area suggests a remote source of inoculum.

In 1911, Freeman and Johnson (24) suggested the possibility of rust migrating from Texas northward. Gassner (25) postulated a similar

migration of spores in South America. Recent field studies[17] indicate that two sources of inoculum are usually present which might account for the primary infection in the spring wheat area, namely, urediniospores in Texas, and local epidemics near barberries in the Northern States. It is not within the scope of this paper to discuss which of these two sources is the more important. It is desired merely to point out that in wind dissemination there is a mechanism which can explain a wide-spread dissemination from either source. Stakman *et al.* (56) recognized the possibility of spores being carried into the air by convection currents, whirlwinds, and other air movements. They exposed glass slides, coated with vaseline, from aeroplanes and caught spores and pollen grains at altitudes up to 10,000 feet, and germinated urediniospores collected at elevations of 1,000 to 7,000 feet. Tremendous numbers of spores were caught on slides exposed over the spring wheat area during the epidemic of 1922. One slide exposed for 2¾ hours at altitudes from 1,000 to 5,000 feet caught more than 3,000 stem-rust urediniospores.

The distance which spores may be carried would seem to depend on the rate of fall of the spores and the velocity of the wind. Buller (10) estimated the rate of fall of basidiospores at 1–3 mm. a second, and McCubbin (36) estimated the rate of fall of urediniospores of *Cronartium ribicola,* which are approximately the size of stem-rust urediniospores, at 8 mm. a second. The rate of fall of urediniospores of *Puccinia graminis* has not been determined, but from what is known of dust movement it seems probable that the ordinary circulation of the air is sufficient to keep them suspended almost indefinitely. Free (23) states that Ulden observed the paths of quartz particles of various sizes allowed to fall freely through a wind blowing with a velocity of approximately 8 miles an hour. It was found that particles averaging 0.75 mm. in diameter fell in a path differing but 10 degrees from the vertical.

Those averaging 0.18 mm. followed a path differing but little from the horizontal. Material 0.08 mm. and less in diameter was blown clear away. Free (23) has tabulated the maximum size of dust particles in the Sirrocco dust blown into Europe from the Sahara desert and found that, in the average dust storms, particles were more than 50 μ and in some cases 200 μ in diameter. The dust which was blown from Australia to New Zealand, 1,500 miles, in 1903, contained particles 30 μ in diameter, approximately the length of a urediniospore. From data obtained by collecting dust in storms occurring previous to 1896, Ulden estimated that 1,225,000,000,000 "mile tons" of dust transfer was accomplished annually in the western States of the United States (23).

[17] Unpublished papers of Stakman *et al.*

These dust storm analogies indicate that the wind is perfectly capable of carrying spores thousands of miles if sufficient spore material is present. According to Meisinger (38), the United States Weather Bureau is just beginning to study the free air trajectories of layers of air higher than the surface, 500–1,000 feet. Is there any direct evidence that winds ever occur which, for example, may transport spores from Texas, where overwintering and early development occur, to the spring wheat area?

The national balloon race, which was held in the Mississippi Valley in 1924, shows how spores may be blown from south to north in this region. Seven balloons started from San Antonio, Tex., in the late afternoon of April 23, 1924. Four of them traveled over 1,000 miles; two landed in southern Minnesota, and two in Iowa. The longest trip was made by Van Orman from San Antonio to Rochester, Minn.

The distance, 1,072 miles, was covered in 44 hours. Peek made somewhat better time than Van Orman, traveling to St. Ansgar, Iowa, a distance of 1,021 miles, in 32 hours and 27 minutes. Air currents at an altitude of 500 to 1,500 meters were used for the larger part of the race, although altitudes up to 5,000 meters were used on one occasion by Van Orman to prevent his balloon from being drawn into a Low. The distances traversed by these balloons, and the rate of travel, are interesting in themselves, but the fact that the balloons started at San Antonio, Tex., and landed in the spring wheat area makes the flights more significant, and suggests that they may furnish a clue to the weather conditions conducive to strong south winds in the western Mississippi Valley.

Jakl (30) has analyzed the weather conditions which existed during the flights. He states, "The winds observed at about the time the balloons took off were steadily southerly, becoming southwesterly at 2,000 meters from Texas, north to North Dakota. Velocities in a southerly current averaged about 30 miles per hour, being the highest about 1,000 meters above the ground, and higher over the northern sections than in the south." These strong southerly winds were attributed by Jakl to the presence of a High over the southeast States, and a Low which extended over the plateau region just east of the Rocky Mountains. This condition, as illustrated in figure 4, persisted throughout April 23 and 24, the days on which the balloons were in the air. The relation of the High in the east and the Low pressure area in the west, to the winds between them, will be fully appreciated when it is recalled that air circulates in a clock-wise direction about a High and a counter-clock-wise direction about a Low.

It has been shown, by exposing slides from an aeroplane, that in some years spores are abundant in the air over central and northern Texas in

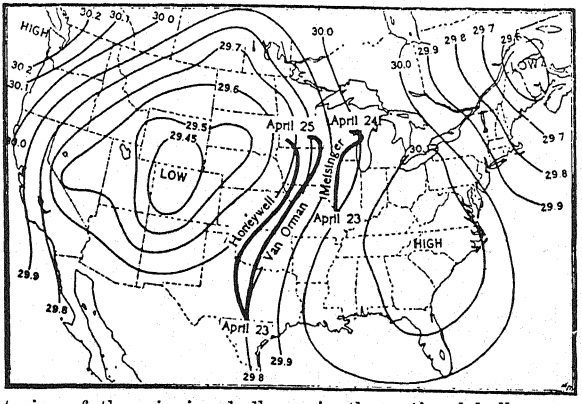


FIG. 4. Trajectories of the winning balloons in the national balloon race of 1924, and the pressure distribution in the evening of the day on which the race started, April 23, 1924. (After Meisinger.)

May. For example, on May 18, 1922, approximately 400 stem rust urediniospores were caught during a flight from San Antonio to Dallas, Texas. These spores were caught at altitudes ranging between 1,000 and 2,000 feet.[18]

To determine whether atmospheric conditions, similar to those mentioned above, exist in late May or early June, the writer examined the Daily Weather Maps for the period from 1901 to 1926. As indicated in table 7, conditions comparable to those shown in figure 4, that is, a High in the east and a Low in the west, separated by approximately straight isobars from the Gulf of Mexico to Canada, occurred from one to several times during the last ten days in May and the first ten days in June in every year for the past 25 years. There is no indication of a correlation between the years in which conditions seemed to be the most favorable for the migration of rust from the south to the north and the years in which epidemics developed in the spring wheat area. But it is clear that southerly winds often sweep up the Mississippi Valley at the time the rust is most plentiful in the south, with sufficient velocity to carry spores from Texas to the spring wheat area in less than three days.

It might be objected that these balloons traveled at altitudes higher than those in which urediniospores are abundant, or that the spores would diffuse to such an extent that only a few would ever reach the spring wheat area. That these objections are not necessarily valid is indicated by the distribution of smoke from Minnesota forest fires in 1918. Lyman (34) summarized the observations on the paths of these smoke clouds and showed

[18] Unpublished papers of Stakman *et al.*

TABLE 7.—*Days on which conditions seemed favorable for winds to blow spores from Texas to the spring wheat area, i.e., with a HIGH in the East and a LOW in the West, during the period May 20 to June 10, from 1901 to 1926 inclusive*

Year	May											June									
	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4	5	6	7	8	9	10
1901	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	+	0
1902	+	0	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0	0	0	0	+
1903	0	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1904	0	+	+	0	+	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	+	0
1905	0	0	0	0	0	0	0	0	+	0	0	0	+	0	+	+	0	0	0	+	0	0
1906	0	+	0	0	0	+	0	0	0	+	0	0	0	+	+	+	0	+	0	0	0	0
1907	0	+	+	0	0	0	0	0	+	0	0	0	0	0	0	0	0	+	+	0	+	0
1908	+	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	+	+	0	0	0	0
1909	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0	0	0	0	0	0	0
1910	0	0	0	0	0	0	+	+	+	0	0	0	0	0	0	0	0	0	0	+	+	0
1911	0	0	0	0	+	0	+	0	0	0	0	0	0	0	+	0	0	0	0	+	+	+
1912	0	0	0	0	0	0	+	+	0	+	0	0	0	0	0	0	0	0	0	+	+	0
1913	0	0	0	0	0	+	0	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0
1914	+	0	0	+	+	+	+	0	+	0	0	0	0	+	+	+	+	+	+	0	0	+
1915	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	+	0	0	0	0	+	0
1916	+	0	0	0	0	+	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0
1917	0	0	0	0	0	+	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	+
1918	0	+	0	0	+	0	0	0	0	0	+	+	0	0	+	0	+	0	0	+	0	0
1919	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0	0	0
1920	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	+	+
1921	+	+	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	0	0
1922	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	+	0	0
1923	0	0	0	0	0	0	+	+	0	0	0	+	+	0	0	0	0	0	0	+	+	0
1924	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0
1925	0	0	+	0	0	0	+	0	0	+	+	+	0	0	0	+	0	0	+	0	0	0
1926	0	0	0	+	+	+	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	+

that they were blown from Minnesota to New York and along the Atlantic Coast to South Carolina in two days. The smoke was not diffused but was disseminated in clouds dense enough to lower the visibility at points along their paths. That it was largely in the lower strata of air is indicated by the fact that the odor of burning wood was recorded in most of the localities where the smoke was seen.

GERMINATION OF UREDINIOSPORES AND UREDINIAL INFECTION

Germination.—Factors affecting the functioning of urediniospores have been studied both in the laboratory and in the field. There is experimental evidence that they will germinate in a wide range of temperature and are

comparatively long-lived especially at low temperatures. It has already been pointed out that the low minimum for spore germination is probably detrimental rather than beneficial, as the minimum temperature for penetration is comparatively high, and germination frequently takes place when the germ tubes are unable to penetrate.

Johnson (31) determined the minimum and maximum temperature for the germination of *Puccinia graminis* at 1.5° to 32° C. Hursh (28) obtained excellent germination at 10°, 20°, and 30° C. with two forms of *P. graminis tritici*. Form 11, of Stakman and Levine (60), which is the more virulent of the two, seemed more tolerant of the higher and lower temperatures than form 27.

De Bary (16) showed that urediniospores retain their viability two months if kept dry. Urediniospores germinated in Bolley's (7) cultures after being exposed to sunlight on glass slides during the month of August. Peltier (46) has shown that urediniospores may retain their viability at a low temperature (5° C.) in moderate relative humidity (49 per cent) for a year or more.

Infection.—Stakman and Levine (59) state, "The most favorable temperature for shortening the incubation period, hastening the maturing and obtaining vigorous infection, appeared to be between 66.5° and 70° F. (19° and 21° C.)." In Peltier's (45, 47) cultures the optimum temperature for the development of stem rust with *Puccinia graminis tritici* forms 3 and 19 of Stakman and Levine on wheat in the seedling, stooling, and jointing stages was between 20° and 25° C. No penetration occurred at temperatures of 15° C. and below, except on Arnautka and einkorn, which were infected at 10° C. The minimum temperature suitable for penetration was about 5° C. lower for form 9 than for form 3. Only a few plants of a limited number of varieties were infected at 30° C. The minimum time for penetration was about 3 hours; fair infection developed after 6 hours, and there was a maximum penetration in 36 hours. The period of incubation was prolonged for nine weeks by submitting the inoculated plants to low temperatures. Both Stakman and Levine (59) and Peltier (47) agree that light is not essential for penetration, but is necessary for rust development, inasmuch as the host plant is dependent upon light for vigorous growth.

Field observations also have been recorded which seem to have a bearing on this problem. Mehta (39) inoculated wheat on successive days in the spring at Cambridge, England, and determined that the incubation period was shortened as the season progressed and the mean temperature increased. At a mean temperature of 40° F. the incubation period was 22 days; at 50.8° it was 15 days; at 54.1°, 12 days; and at 66.5°, 9 days. Tehon and

Young (62), in 1923, noted a coincidence between the occurrence of primary infection in Illinois and a mean temperature of 71.5° F. preceded by abundant rainfall. They believed this to be a causal relationship. Beauverie (2) noted that no stem rust developed in the experimental gardens at Limagne and Clermont in 1921, while it was abundant in 1922. This he attributed to the fact that in 1921 there was no rain from the first ten days in June until harvest, while rainfall was frequent and often abundant during the same period in 1922.

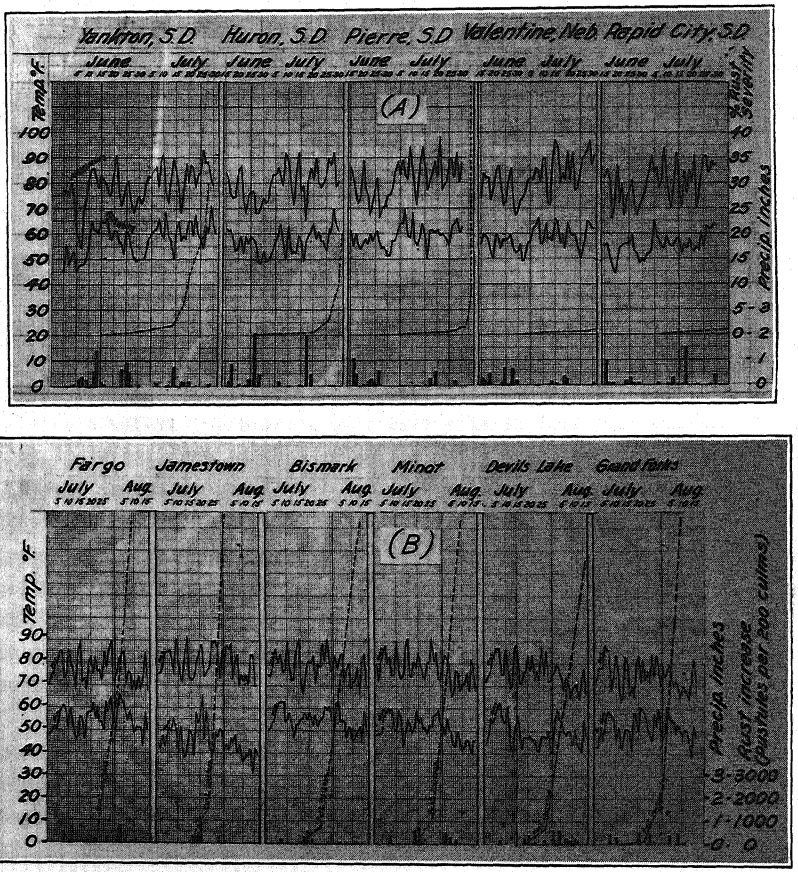


FIG. 5–A. Relation of stem rust development to the temperature range and amount of precipitation at Valentine, Nebraska, and at Yankton, Huron, Pierre, and Rapid City, South Dakota, in June and July, 1924.

B. Relation of stem rust development to the temperature range and the amount of precipitation at Fargo, Jamestown, Bismarck, Minot, Devils Lake, and Grand Forks, North Dakota, in July and August, 1924.

In 1924, repeated observations were made to determine the relation of weather conditions to the rate of development of stem rust at several points in Minnesota, South Dakota, and North Dakota.[19] Figure 5, A and B illustrates the rust development, together with the temperature range and the amount of precipitation at the different stages, in North Dakota and South Dakota. Two different methods were used for recording the rust; in South Dakota the percentage of rust severity, and in North Dakota the number of pustules on 200 culms. As indicated in the figures, the inoculum increased at so tremendous a rate as the season progressed that it complicated the problem to such an extent that no definite conclusions could be drawn.

THE DEVELOPMENT OF DESTRUCTIVE EPIDEMICS

Destructive epidemics occur periodically in the spring wheat area. Several attempts have been made to determine the relation of seasonal weather conditions to the development of these epidemics. Some investigators regard the effect of weather on the rate of growth of the host as the most important; others emphasize the effect of weather on the development of the pathogene.

Certainly both must be considered, since a destructive epidemic involves a race between the pathogene and the host.

There can be little doubt that the vigor and rate of growth of wheat is affected by seasonal variations in temperature and precipitation. Hutcheson and Quantz (29) grew small grains at 58°, 62°, 65°, and 75° F. and considered the optima to be 62° F. for wheat (Fulcaster), 65° to 75° F. for oats (Culberson), 58° to 62° F. for barley (Union Winter), and 62° F. for rye (Abruzzes). Peltier (47), as a result of experiments in the greenhouse, considered 15° to 20° C. the best temperatures for the development of the seedling, stooling, jointing, and heading stages of several wheat varieties, including certain spring wheats. In his experiments, temperatures below 10° C. resulted in slow growth, and likewise temperatures above 25° C. inhibited growth.

Five years' records[20] of the date of sowing and heading in the rust nurseries at St. Paul, given in table 9, indicate that in 1924 the period between sowing and heading was delayed considerably by low temperatures in May and June.

Blair (3, 4, and 5) has shown that low temperature during the growing season is correlated with high yields of winter wheat in Ohio and spring

[19] In North Dakota the development of the rust was observed by Frolik, while in South Dakota and Nebraska observations were made by Butler.

[20] These were furnished by Dr. Olaf S. Aamodt, formerly Associate Pathologist, Office of Cereal Crops and Diseases, U. S. Department of Agriculture.

TABLE 9.—*The relation of mean temperatures to the length of the growing period of Marquis wheat in the stem rust nursery at St. Paul, Minnesota, during the period 1920 to 1924 inclusive*

Year	Date of sowing	Date of heading	No. days from sowing to heading	Mean departures from normal temperatures[a]		
				May	June	Average
1920	April 29	June 28	59	+0.8	+0.6	+0.70
1921	April 25	June 24	59	+1.6	+6.1	+0.37
1922	April 24	June 21	57	+4.6	+1.2	+2.90
1923	April 25	June 25	60	+0.7	+2.9	+1.80
1924	April 24	July 8	75	−8.1	−3.5	−5.80

[a] The normal temperatures as given in the Monthly Weather Review are 58.2° F. for May, and 67.4° F. for June.

wheat in North and South Dakota. Low mean temperatures in June and abundant precipitation in May and June were correlated with high yields of spring wheat in North and South Dakota.

Sorauer (54) reviewed the earlier European literature on the relation of weather to the development of cereal rusts, and was of the opinion that weather conditions, such as sudden changes in temperature, would predispose the host plants to rust.

Carleton (12) attributed the abundance of rust in the spring wheat area in 1904 to: "(1) The fact that there was an unusual quantity of moisture just at the proper time for rust to do the most damage to the crop, and (2) the unusual delay in ripening the grain." He considered the period between the date of blossoming and the date of ripening the critical time, because rust was plentiful in the field at that time and because rainfall tended to make more succulent growth and delay maturity.

Freeman and Johnson (24) also emphasize the importance of the grain maturing as early as possible to escape damage from rust. They believe the critical period for wheat with respect to attacks of *Puccinia graminis* is heading time, a period of ten days for any one locality. They state, "If for any reason this period is delayed or lengthened, the number of urediniospores falling on each plant is very considerably increased. Infections have a longer time in which to develop and the danger of an epidemic is imminent. They analyzed the relation of precipitation and temperature to the development of rust in the years 1903, 1904, and 1905. During these years there was least rust in 1903, a severe epidemic in 1904, and a moderate amount of rust in 1905. The greatest precipitation occurred in 1905,

whether we consider a seven-months' period preceding harvest, a three-months' growing period, or the month containing the "critical period." Since 1904 was the epidemic year, precipitation was not considered to be the most important factor. From the temperature records they conclude, "That although the general average of temperatures for the whole area for seven-month and three-month periods (preceding harvest) in 1903, 1904, and 1905 were not much different, still in those states where the rust was most severe in 1904, namely, Minnesota, North Dakota, South Dakota, Iowa and Wisconsin, temperatures for the seven-month period averaged much lower in 1904 than in 1905, and for the three-month period averaged about the same as in 1905.

"During the one-month period temperatures were consistently subnormal in 1904, averaging 2.67° below normal for the whole region; temperatures were 3.5° below normal over South Dakota, North Dakota, Minnesota, and Wisconsin; this average being considerably lower than that of either 1903 or 1905. It is seen, then, that the unusual low temperature over this region was a very important factor, if not the determining factor, for the prevalence of rust in 1904. Low temperatures made the crop as a whole late." Bolley (7) also believed that dew and cool temperatures favored the development of rust in 1904.

Cotton (14) is inclined to stress the effect of weather on the pathogene. He accounted for a less serious attack of stem rust in southern England in 1920 than in 1919 on the basis of, first, the infection as a whole being less extensive, and second, the cool weather previous to ripening tending to check the growth of the mycelium in the tissues, and to prevent excessive evaporation following the splitting of the cortical tissues of the stem.

Walster (64) divided the growing season for wheat at Fargo, North Dakota, into nine periods of 10 days each, and compared the maximum and minimum temperatures and precipitation of each period, in five epidemic years, with those of similar periods in five non-rust years. He concluded that high temperature and abundant precipitation were correlated with the occurrence of rust epidemics.

Levine (33) found an apparent positive correlation between the combined mean temperature and precipitation in the month of June and the percentage of rust at the end of the growing season, in "uniform rust nurseries" at St. Paul, Minn., Mandan and Dickson, N. Dak., and Akron, Colo., in the years 1919 to 1923 inclusive. An attempt also was made to determine if there was a correlation between mean temperature and total precipitation during the last two months of the growing season and stem rust infection at harvest time, on three varieties of wheat growing in numerous rust nurseries throughout the Mississippi Valley and in Mani-

toba, Saskatchewan, and Alberta, Canada. Correlations were made with 264 varietal units in 89 nursery years. A coefficient of correlation of $+0.283 \pm 0.038$ was obtained for temperature and rust infection, and $+0.336 \pm 0.037$ for total precipitation and rust infection; at the same time there was no apparent correlation between temperature and rainfall. No infection whatever occurred where the total rainfall was below 2 inches or where the mean temperature was less than 60° F. Barring one exception, not more than 40 per cent rust occurred at or below 62° F. From 90 to 100 per cent rust was found only at the mean temperature range of 66° to 72° F., coupled with a total rainfall exceeding 2.5 inches. The multiple correlation between these two weather conditions and the severity of stem rust infection was +0.4239. Levine states, "These results would justify the conclusion that in the final analysis weather conditions during the last two months of the growing season play only a minor, even if very important, part in the intenseness of stem rust epidemics. "Stakman and Lambert (58) briefly summarized observations which point toward a correlation between warm growing seasons and epidemics in the spring wheat area.

Epidemics in the Spring Wheat Area

The writer has studied the relation of seasonal weather conditions to the occurrence of destructive epidemics in the spring wheat area during the period 1904 to 1925 inclusive. The historical method was used. The months of May, June, and July were considered most important in the spring wheat area because these months are the growing season for spring wheat, and because barberries become infected in May, and rust is spreading from barberries and multiplying in grain fields in June and July. The following factors were considered: mean monthly temperatures, total monthly temperatures, total monthly precipitation, the number of rainy days, and the number of clear days. All weather records, unless otherwise stated, were taken from Climatalogical Data of the United States Department of Agriculture Weather Bureau. Since temperature seems to be the most important factor, its relation to epidemics was also studied in the Southern and Central States. The first step was to classify the years according to the amount of rust which developed in them.

The epidemic of 1904 first focussed attention on stem rust as a limiting factor in the production of grain in the spring wheat area. Carleton (12) characterized this epidemic as follows: "Probably the most severe attack of rust ever known in that region, certainly the most severe in the last 20 or 25 years, occurred during that season." Since that time state patholo-

gists and other cereal investigators have kept fairly complete records[21] of the relative severity of epidemics.

It is apparent from these records that, during the past quarter century, the worst epidemics occurred in 1904, 1911, 1916, 1919, 1920, and 1923. There was comparatively little rust in 1907, 1909, 1910, 1912, 1913, 1915, 1918, and 1924. During the remaining years, 1905, 1906, 1908, 1914, 1917, 1921, 1922, and 1925, the rust was intermediate or indeterminate. For purposes of comparison, the first series will be designated epidemic years, the second series non-epidemic, and the third series intermediate. The approximate differences in the amount of rust from year to year are illustrated diagrammatically in Figure 6. In most of the comparisons the intermediate

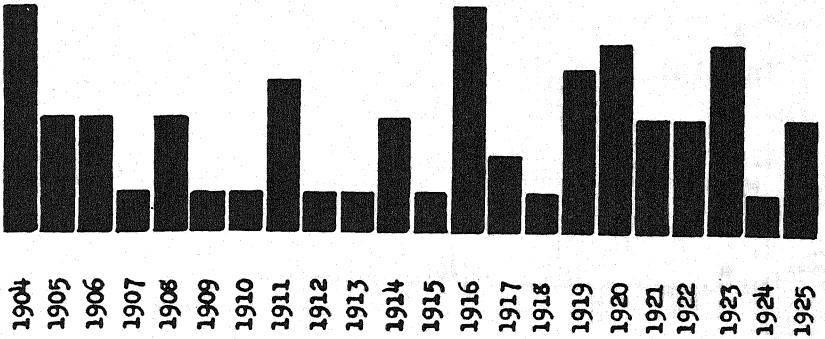


FIG. 6. Comparative severity of stem rust epidemics in the spring wheat area during the period 1904 to 1925, inclusive.

years are left out of consideration because it was thought that there was not enough difference between the amount of rust in these years and in the epidemic or non-epidemic years.

Stakman and Lambert (58) have already pointed out that the average mean monthly temperatures in Minnesota, North Dakota, and South Dakota during the three months considered were above normal for the epidemic years and below normal for the non-epidemic years.

They also showed that the amount of departure from normal seems to indicate a correlation between temperature in these three states and the occurrence of epidemics. The three coolest years were non-epidemic, and the five warmest years were epidemic or intermediate years. In these states, epidemics did not develop in seasons during which the average mean monthly temperatures in May, June, and July were below 61° F. Nor did

[21] This material was kindly placed at the writer's disposal by Dr. R. J. Haskell, Associate Pathologist, in charge of Plant Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

non-epidemic seasons occur when the average mean monthly temperatures were above 64° F. Both epidemic and non-epidemic years were included in seasons of medium average temperature (61°–64° F.). The comparative meteorological data upon which these conclusions were based are given in tables 10, 11, 12, and 13.

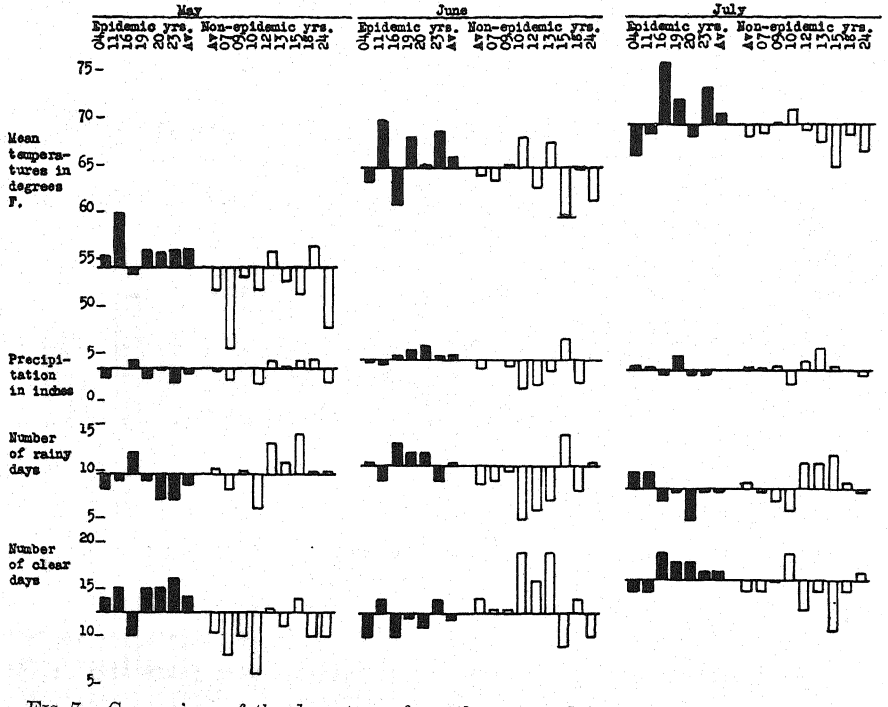


FIG. 7. Comparison of the departures from the normal Minnesota weather conditions which occurred during stem rust epidemic years and non-epidemic years in May, June, and July, from 1904 to 1925 inclusive.

At the suggestion of Dr. J. Arthur Harris,[22] an attempt was made to determine the mathematical significance of this apparent correlation between mean temperature and rust epidemics. The χ^2 test for goodness of fit was used.[23] The data are given in tables 14 and 15, and compared in table 15 with the distribution of years calculated on the basis of random sampling. The results indicate that deviations from the calculated distribution, as

[22] Professor of Botany, University of Minnesota.

[23] Following the usage in a recent paper in "Phytopathology" by C. H. Goulden, K. W. Neatby and J. N. Welch, the value of P was taken from Pearson's tables in the column under $n' = 5$. This takes into consideration the number of degrees of freedom as suggested by Fisher.

TABLE 10.—*Comparative meteorological data for May, June, and July, the critical months for the development of stem rust in Minnesota*

Year	May				June				July			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	55.4	2.43	8	14	63.2	4.26	11	10	66.0	3.96	10	15
1905	52.6	5.54	12	10	63.0	6.41	14	9	67.3	4.12	8	15
1906	53.7	5.58	14	9	63.7	4.55	12	10	68.3	2.93	8	17
1907	45.5	2.14	8	9	63.3	4.31	9	13	68.2	3.57	8	15
1908	53.9	6.31	12	11	62.5	6.35	12	10	69.4	3.21	8	18
1909	53.2	3.36	10	10	65.0	3.53	10	13	69.2	3.84	7	16
1910	51.6	1.58	6	16	67.8	1.39	5	19	70.6	1.94	6	19
1911	59.8	3.48	9	15	69.7	3.79	9	14	68.2	3.61	10	15
1912	55.9	4.13	13	10	62.5	1.66	6	16	68.5	4.30	11	13
1913	52.7	3.53	11	11	67.4	3.08	7	19	67.3	5.56	11	15
1914	57.6	2.89	9	14	64.6	8.34	15	10	72.4	2.48	6	18
1915	51.4	4.13	14	8	59.4	6.52	14	9	64.7	3.95	12	11
1916	53.5	4.27	12	10	60.8	4.93	13	10	75.5	2.85	7	19
1917	52.0	1.94	5	19	60.7	3.26	11	12	70.8	3.33	9	18
1918	56.4	4.39	10	10	64.4	1.99	8	14	68.0	3.35	9	15
1919	56.0	2.37	9	15	67.9	5.32	12	12	71.7	4.83	8	18
1920	55.6	3.14	7	15	64.9	5.95	12	11	67.9	2.88	5	18
1921	57.1	3.47	10	14	70.3	2.90	7	17	74.2	3.62	9	17
1922	60.2	3.07	10	10	66.4	3.00	9	14	67.3	2.26	8	14
1923	56.0	1.94	7	16	68.4	4.62	9	14	72.9	2.82	8	17
1924	47.7	1.85	10	12	61.1	4.27	11	10	66.4	2.77	8	17
1925	52.8	1.55	5	18	63.5	6.91	16	10	67.5	2.99	8	17
Averages	54.12	3.32	9.59	12.5	64.56	4.42	10.54	12.5	69.19	3.42	8.36	16.2

great or greater than those obtained, would be expected on the basis of random sampling only 15 times out of 100. In other words, the odds are 6 to 1 that the apparent correlation between warm growing seasons and destructive epidemics is not due to chance.

If a causal relationship is assumed, three noteworthy exceptions to the general rule must be explained; first, 1910 was a non-rust year in spite of the fact that a high temperature prevailed in June and July in Minnesota, North Dakota, and South Dakota. This was probably due to the fact that 1910 was the driest year in the 22 years considered. Second, a severe epidemic occurred in 1916 in spite of a cool May and June. This may be partially explained by the abnormally high temperature which prevailed in July, 1916. Third, an epidemic also developed in the moderately cool season of 1904. This seems to be a real exception to the rule, indicating that, in certain seasons, other factors may be favorable enough to induce an epidemic in cool seasons. (See figures 7, 8, and 9.)

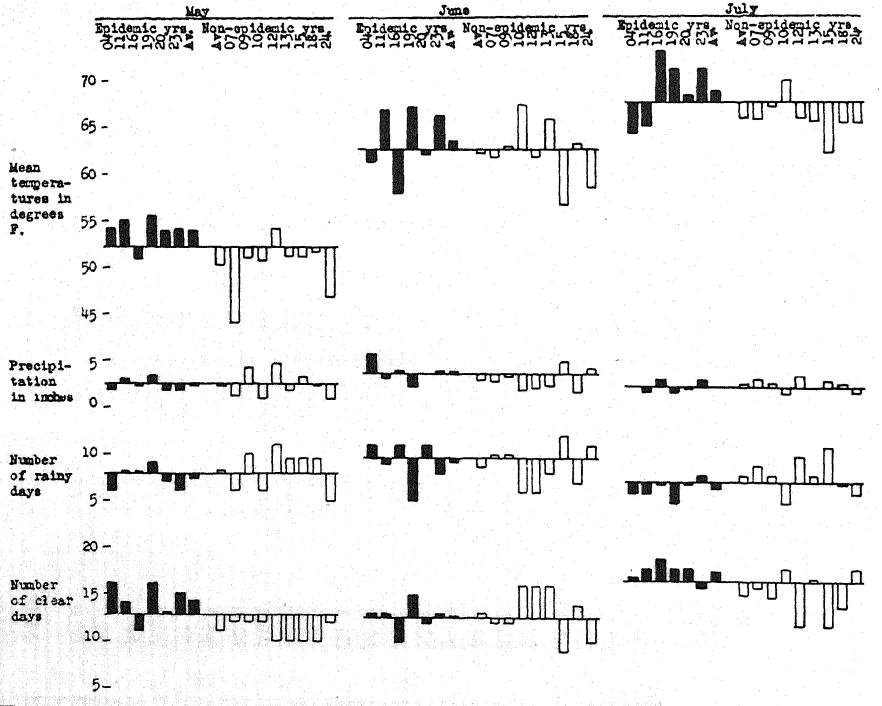


FIG. 8. Comparison of the departure from the normal North Dakota weather conditions which occurred during stem rust epidemic years and non-epidemic years in May, June, and July, from 1904 to 1925 inclusive.

TABLE 11.—*Comparative meteorological data for May, June, and July, the critical months for the development of stem rust in North Dakota*

Year	May				June				July			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	54.2	1.76	6	16	61.4	5.77	11	13	64.4	2.49	6	17
1905	50.1	3.05	8	12	59.7	4.52	12	10	65.6	3.90	8	17
1906	50.8	4.63	12	9	62.0	4.06	11	13	66.9	1.97	11	13
1907	44.0	1.11	6	12	61.9	2.85	10	12	65.8	3.14	9	16
1908	50.8	2.97	8	13	60.4	3.37	10	12	68.6	2.10	6	19
1909	51.0	4.29	10	12	62.9	3.21	10	12	67.1	2.89	8	15
1910	50.7	0.96	6	12	67.3	1.83	6	16	70.0	1.60	5	18
1911	55.0	2.88	8	14	66.9	3.12	9	13	65.1	1.82	6	18
1912	54.2	4.52	11	10	61.8	2.05	6	16	66.0	3.54	10	12
1913	51.2	1.68	9	10	65.8	2.35	8	16	65.6	2.30	8	17
1914	55.2	2.12	8	16	62.2	6.26	13	9	72.1	2.31	6	19
1915	51.1	3.16	9	10	56.7	5.00	12	9	62.3	3.01	11	12
1916	51.0	2.22	8	11	58.0	3.89	11	10	73.1	3.16	7	19
1917	51.2	0.30	2	20	59.7	2.09	8	13	70.9	1.66	7	17
1918	51.7	2.30	9	10	63.3	1.63	7	14	65.5	2.71	7	14
1919	55.5	3.25	9	16	67.2	2.31	5	15	71.2	1.81	5	18
1920	53.9	1.74	7	13	62.2	3.62	11	12	68.3	2.16	7	18
1921	52.8	2.46	9	13	68.2	3.51	9	14	71.4	3.07	7	17
1922	56.3	3.11	12	10	63.5	3.78	9	15	65.6	2.43	8	16
1923	54.1	1.77	6	15	66.1	3.82	8	13	71.2	3.14	8	16
1924	46.9	0.79	5	12	58.6	4.17	11	10	65.5	1.95	6	18
1925	53.6	1.44	4	17	61.5	6.07	14	11	66.7	1.34	6	18
Averages	52.05	2.38	7.81	12.86	62.6	3.60	9.59	12.63	67.67	2.47	7.36	16.54

TABLE 12.—*Comparative meteorological data for May, June, and July, the critical months for the development of stem rust in South Dakota*

Year	May				June				July			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	57.5	2.46	8	13	64.5	4.07	11	14	68.7	2.67	8	19
1905	53.0	5.84	12	11	64.4	5.78	12	10	68.5	3.63	8	16
1906	56.6	4.95	11	12	63.9	3.48	8	15	69.6	1.45	6	20
1907	48.9	3.83	8	12	64.2	3.90	9	15	69.9	4.35	11	16
1908	54.8	4.65	9	13	63.7	5.32	12	13	71.6	3.20	7	20
1909	54.8	4.85	10	11	66.9	4.10	11	13	71.0	3.07	8	18
1910	53.4	1.63	7	15	68.3	2.27	7	16	73.4	1.99	7	19
1911	60.3	1.70	6	15	73.4	1.85	6	17	71.0	2.08	7	17
1912	57.1	2.35	8	12	64.8	1.49	5	16	70.9	3.86	10	14
1913	54.4	3.48	11	9	69.6	2.50	7	16	71.1	2.43	14	16
1914	57.3	3.16	10	12	67.6	5.01	12	12	75.8	2.09	5	19
1915	52.0	3.64	12	7	60.3	5.34	13	10	65.3	5.89	13	11
1916	54.5	4.13	10	13	61.0	3.74	12	12	76.9	2.40	7	20
1917	51.8	3.16	8	14	62.7	2.08	7	15	74.3	1.47	5	21
1918	57.5	3.56	11	12	68.2	2.54	8	16	70.4	3.23	8	16
1919	56.2	2.79	8	17	68.7	3.13	8	15	74.9	2.85	6	19
1920	55.6	4.79	10	11	64.9	4.79	12	11	71.1	2.30	7	19
1921	56.5	3.21	11	10	72.5	2.10	6	18	75.0	3.41	8	18
1922	57.9	3.59	11	10	68.4	3.72	9	18	69.2	3.69	10	15
1923	55.4	2.40	9	12	67.0	4.53	11	13	74.6	3.45	9	15
1924	50.8	0.86	6	11	62.6	4.88	14	10	69.2	1.68	6	20
1925	56.4	1.52	5	15	65.5	5.66	12	13	71.5	1.74	6	19
Averages	55.12	3.16	9.13	12.13	66.05	3.74	9.63	14.0	71.54	2.86	8.0	17.59

Comparisons also were made to determine the relation of mean temperature in Texas, Oklahoma, Kansas, Nebraska, and Iowa, to the occurrence of epidemics in the spring wheat area. The results of these comparisons are given in figures 10 and 11, which are based on meteorological data given in tables 16 to 21 inclusive. It is evident that no striking correlation exists.

Number of clear days.—There seems to have been a tendency for epidemics to develop in years having the greatest number of clear days in the growing season. This may be attributed to a possible correlation between high temperatures and clear days. There is also a possibility that clear days are correlated with the formation of dew at night.

Rainfall and number of rainy days.—As indicated in figures 8, 9, and 10, there was no apparent correlation between the amount of precipitation and the development of epidemics with the possible exception of the effect of drought in 1910.

Dew.—The United States Weather Bureau does not record the formation of dew, nor has a systematic survey of the condition been included in

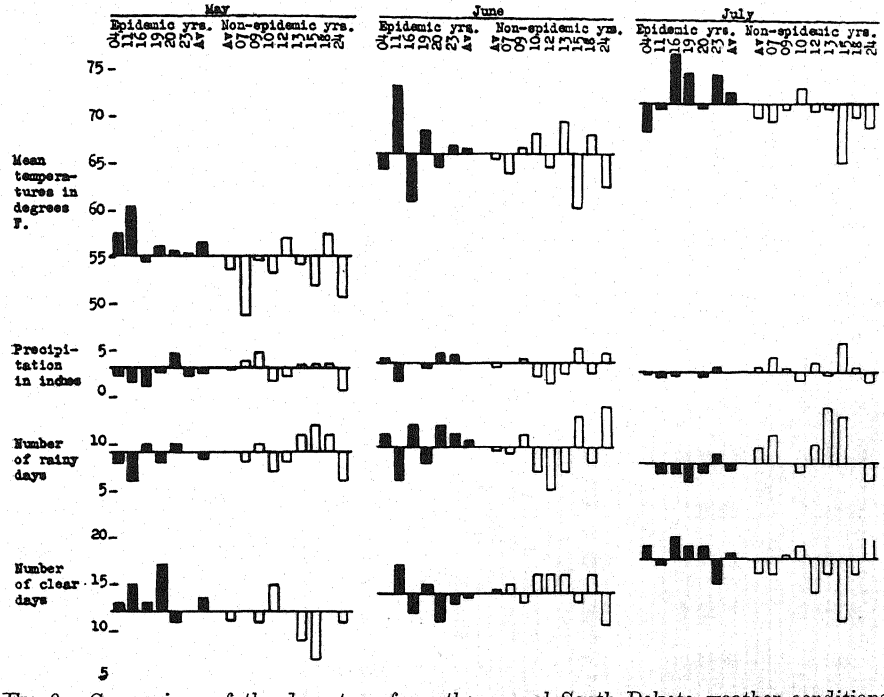


FIG. 9. Comparison of the departure from the normal South Dakota weather conditions which occurred during stem rust epidemic years in May, June, and July, from 1904 to 1925 inclusive.

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TABLE 13.—*A comparison of the deviations, of the average mean monthly temperatures of the stem rust epidemic years with those of the non-epidemic years, from the average for the 22-year period 1904 to 1925, inclusive*

States	May		June		July		Average	
	Epidemic	Non-epidemic	Epidemic	Non-epidemic	Epidemic	Non-epidemic	Epidemic	Non-epidemic
Minnesota	+1.95	−2.3	+1.22	−0.73	+1.14	−1.32	+1.44	−1.45
North Dakota...	+1.95	−1.9	+1.03	−0.3	+1.18	−1.75	+1.39	−1.32
South Dakota...	+1.48	−1.49	+0.58	−0.39	+1.34	−1.35	+1.13	−1.08
Average	+1.79	−1.89	+0.94	−0.47	+1.22	−1.48	+1.32	−1.28

TABLE 14.—*Correlation between the average of the mean monthly temperatures for the growing season (May, June, and July) in the spring wheat area (Minnesota, North Dakota, and South Dakota) from 1904 to 1925 inclusive, and the development of stem rust epidemics in that area. Also the expected distribution calculated on the basis of random sampling*

Temperature (degrees Fahrenheit)	Epidemic	Uncertain	Non-Epidemic	
58–61		1	3	4
61–64	3	5	5	13
64–67	3	2		5
	6	8	8	22

TABLE 15.—*Classification of years during the period 1904 to 1925 inclusive on the basis of the occurrence of epidemics and the average of the mean monthly temperatures in May, June, and July, and the goodness of fit to the expected distribution calculated on the basis of random sampling*

Class	Number of years		O − C	$\frac{(O-C)^2}{C}$
	Observed (O)	Calculated (C)		
Epidemic ..... 58°–61° F.	0	$\frac{4\times6}{22}=1.0909$	−1.0909	1.0908
Uncertain ..... 58°–61° F.	1	$\frac{4\times8}{22}=1.4545$	−0.4545	0.1419
Non-epidemic ..... 58°–61° F.	3	$\frac{4\times8}{22}=1.4545$	1.5455	1.6421
Epidemic ..... 61°–64° F.	3	$\frac{13\times6}{22}=3.5455$	−0.5455	0.0839
Uncertain ..... 61°–64° F.	5	$\frac{13\times8}{22}=4.7273$	0.2727	0.0157
Non-epidemic ..... 61°–64° F.	5	$\frac{13\times8}{22}=4.7273$	0.2727	0.0157
Epidemic ..... 64°–67° F.	3	$\frac{5\times6}{22}=1.3636$	1.6364	1.9638
Uncertain ..... 64°–67° F.	2	$\frac{5\times8}{22}=1.8182$	0.1818	0.0181
Non-epidemic ..... 64°–67° F.	0	$\frac{5\times8}{22}=1.8182$	−1.8182	1.8181
Total	22	22.0000	Σ 7.8182	Σ 6.7901
P = .149		$\chi^2 = 6.7901$		

TABLE 16.—*Comparative meteorological data for March, April, May, and June, the critical months for the development of stem rust in Texas*

Year	March				April				May				June			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	64.8	1.05	2	15	66.3	2.98	6	14	72.5	4.56	6	17	79.9	4.28	6	15
1905	61.4	4.29	8	11	64.5	6.32	7	15	74.8	4.82	7	12	80.5	4.64	7	16
1906	54.0	1.72	5	14	66.3	2.67	6	11	73.3	2.98	6	14	80.6	2.71	6	18
1907	67.3	1.64	3	15	62.5	2.42	5	12	68.3	6.73	9	10	79.8	1.71	3	18
1908	64.3	1.65	4	13	67.0	5.08	9	11	73.5	5.69	5	14	81.1	2.48	4	15
1909	59.5	1.08	4	15	66.1	1.55	4	15	72.9	3.06	6	13	80.9	3.37	6	16
1910	64.4	1.55	3	19	65.9	2.59	5	17	72.4	3.91	6	13	80.6	1.90	4	18
1911	63.1	2.19	5	14	66.4	4.88	9	9	73.2	2.13	4	18	83.7	0.88	2	21
1912	53.1	2.75	7	9	65.8	2.87	6	12	74.5	2.28	5	18	77.4	3.71	5	16
1913	55.2	1.69	4	16	64.8	2.02	3	20	73.5	2.55	4	18	78.0	3.61	7	11
1914	56.3	2.45	5	16	64.8	4.28	6	12	71.3	7.68	11	9	81.1	1.47	3	19
1915	49.1	1.83	5	12	65.8	5.82	8	10	72.7	2.47	5	15	81.2	2.24	4	18
1916	63.7	0.64	1	22	64.2	3.44	5	14	74.4	3.80	5	18	81.7	2.17	4	18
1917	59.5	0.94	4	16	65.5	2.17	4	17	69.1	2.76	6	15	81.2	0.80	2	21
1918	62.8	1.35	3	15	65.1	3.99	6	14	74.6	2.40	4	14	83.3	2.45	5	17
1919	57.3	2.85	5	14	65.6	2.55	5	16	70.7	5.31	9	12	76.3	5.80	10	10
1920	57.3	1.33	3	16	65.0	1.05	3	19	74.3	5.11	7	12	78.1	3.58	7	16
1921	64.5	2.80	5	13	64.3	3.68	6	17	73.6	1.94	4	16	79.4	6.58	9	13
1922	57.7	3.69	5	16	67.6	6.26	7	10	75.0	4.46	8	14	79.5	3.55	6	15
1923	54.8	3.02	6	15	66.2	4.16	8	12	74.0	1.97	5	18	81.0	2.96	6	16
1924	53.6	2.26	6	14	65.6	2.83	4	13	70.0	4.35	7	13	82.1	2.12	4	21
1925	63.1	0.65	2	18	71.4	2.06	4	15	73.7	2.65	5	16	83.4	1.41	4	16
ages ...	59.40	1.97	4.32	14.91	65.75	3.42	5.73	13.86	71.92	3.80	6.09	14.50	80.49	2.93	5.18	16.54

TABLE 17.—*Comparative meteorological data for May, June, and July, the critical months for the development of stem rust in Oklahoma*

Year	May				June				July			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	67.4	4.95	9	14	75.1	8.37	8	14	79.6	3.86	7	20
1905	69.4	7.51	11	13	78.5	1.88	5	18	77.7	5.50	9	16
1906	68.6	3.22	7	13	75.0	4.18	8	16	76.5	5.26	9	13
1907	61.9	5.02	10	13	75.1	5.34	8	16	80.7	2.13	4	22
1908	67.8	8.45	9	15	75.2	8.85	11	14	77.7	3.15	6	17
1909	67.1	5.49	6	17	76.5	3.45	9	15	83.7	2.13	4	21
1910	65.5	3.90	10	13	76.5	1.48	5	17	83.0	2.15	4	20
1911	71.3	2.61	6	18	84.0	1.12	3	23	80.7	5.34	10	13
1912	70.9	2.95	6	21	73.7	4.60	7	18	83.2	1.40	3	22
1913	70.4	3.50	6	17	76.0	2.62	7	12	83.8	2.38	4	20
1914	66.9	5.17	11	10	81.2	0.99	3	20	84.6	1.63	4	19
1915	65.8	6.01	9	15	74.2	7.12	11	12	78.5	2.92	6	19
1916	70.1	2.13	5	17	75.4	6.60	10	13	82.9	0.76	2	24
1917	62.5	2.32	6	13	76.8	2.09	4	21	83.3	2.57	6	19
1918	71.3	4.05	6	16	81.4	3.40	6	12	82.1	1.38	4	19
1919	66.0	4.61	11	9	74.5	4.29	10	11	81.7	1.75	4	22
1920	69.0	5.33	9	13	75.7	2.70	6	17	80.7	3.27	6	17
1921	70.0	2.04	6	14	76.2	8.05	14	8	81.2	2.49	5	19
1922	69.1	5.72	11	12	77.8	1.74	5	17	81.7	4.08	6	20
1923	67.0	6.63	10	13	77.2	5.01	11	16	83.2	1.10	3	20
1924	63.8	2.44	6	16	80.5	3.35	6	19	79.6	2.89	7	17
1925	68.0	2.23	5	17	82.5	2.13	5	19	83.7	3.95	9	14
Averages	67.71	4.37	7.95	14.50	77.22	4.06	7.36	15.81	81.35	2.82	5.55	18.77

our observations. Judging from personal experience, the writer is of the opinion that in the spring wheat area the formation of dew at night is the rule rather than the exception during June, July, and August. At St. Paul, Minn., heavy dew is usually present on grass at 10 p. m. and persists until 8 or 9 a. m. This would allow ample time for the penetration of the rust organism. The average relative humidity at 8 a. m. in July is higher in the Red River Valley in Minnesota than in any other part of the Mississippi Valley except the coast of the Gulf of Mexico. This high morning humidity is probably correlated with the occurrence of dew, and it may be more than a coincidence that grain usually suffers greater loss from stem rust in the Red River Valley, where the morning humidity is higher than in any other district in the Mississippi Valley.

During the rust survey in August, 1923, the writer, with J. J. Christensen, D. L. Bailey, and W. P. Fraser, found stem rust of 95 per cent severity

TABLE 18.—*Comparative meteorological data for May and June, the critical months for the development of stem rust in Kansas*

Year	May				June			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	62.4	5.43	11	13	70.4	6.57	12	11
1905	63.1	4.42	10	13	75.5	3.75	7	15
1906	65.0	2.49	8	14	71.8	3.67	7	16
1907	58.3	2.37	8	13	72.0	4.83	10	15
1908	63.8	5.07	11	13	72.1	7.91	13	11
1909	62.2	3.66	8	14	73.1	5.46	12	11
1910	60.0	4.83	12	11	72.2	1.88	6	16
1911	66.7	2.66	8	14	80.5	0.64	3	21
1912	66.8	2.87	6	18	69.3	4.02	9	15
1913	66.7	2.90	7	16	74.2	2.45	7	13
1914	64.3	3.39	9	11	78.2	3.96	8	18
1915	60.3	6.85	12	14	69.2	6.46	13	9
1916	64.2	3.32	8	16	70.3	6.01	10	15
1917	57.5	3.50	9	12	72.8	2.11	5	21
1918	67.6	4.13	9	15	78.4	1.31	4	21
1919	61.3	2.97	9	11	72.3	4.36	10	14
1920	63.2	3.07	9	8	72.8	2.93	7	16
1921	65.5	2.61	6	14	74.4	5.00	11	12
1922	64.6	3.97	11	13	75.2	2.33	6	20
1923	61.3	5.75	12	12	73.0	6.10	11	13
1924	58.1	2.12	7	14	74.1	2.17	7	16
1925	62.9	2.16	7	15	77.6	4.03	8	18
Averages	62.99	3.66	8.95	13.4	73.6	3.99	8.45	15.3

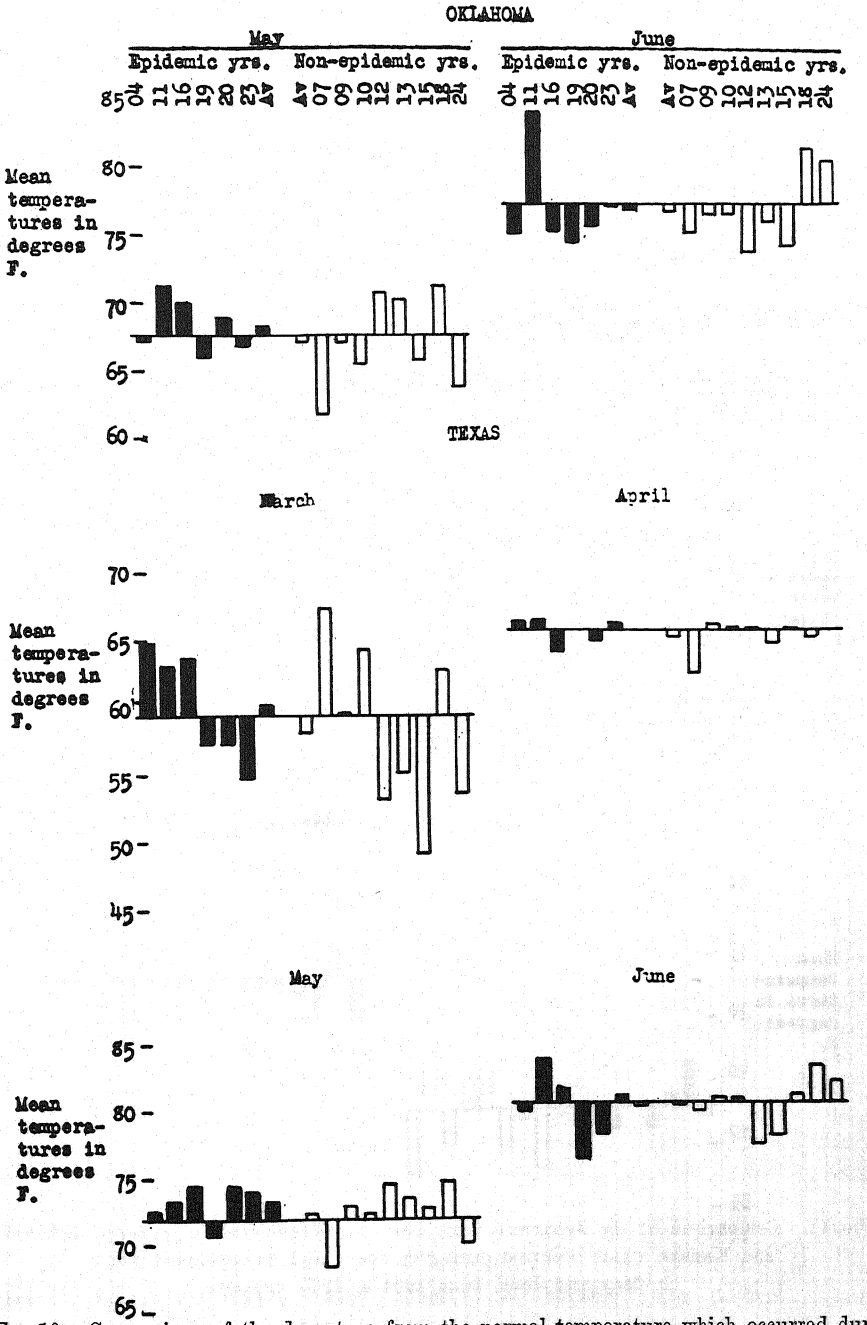


FIG. 10. Comparison of the departure from the normal temperature which occurred during the epidemic and non-epidemic years in Oklahoma in May and June, and in Texas in March, April, May and June, from 1904 to 1925 inclusive.

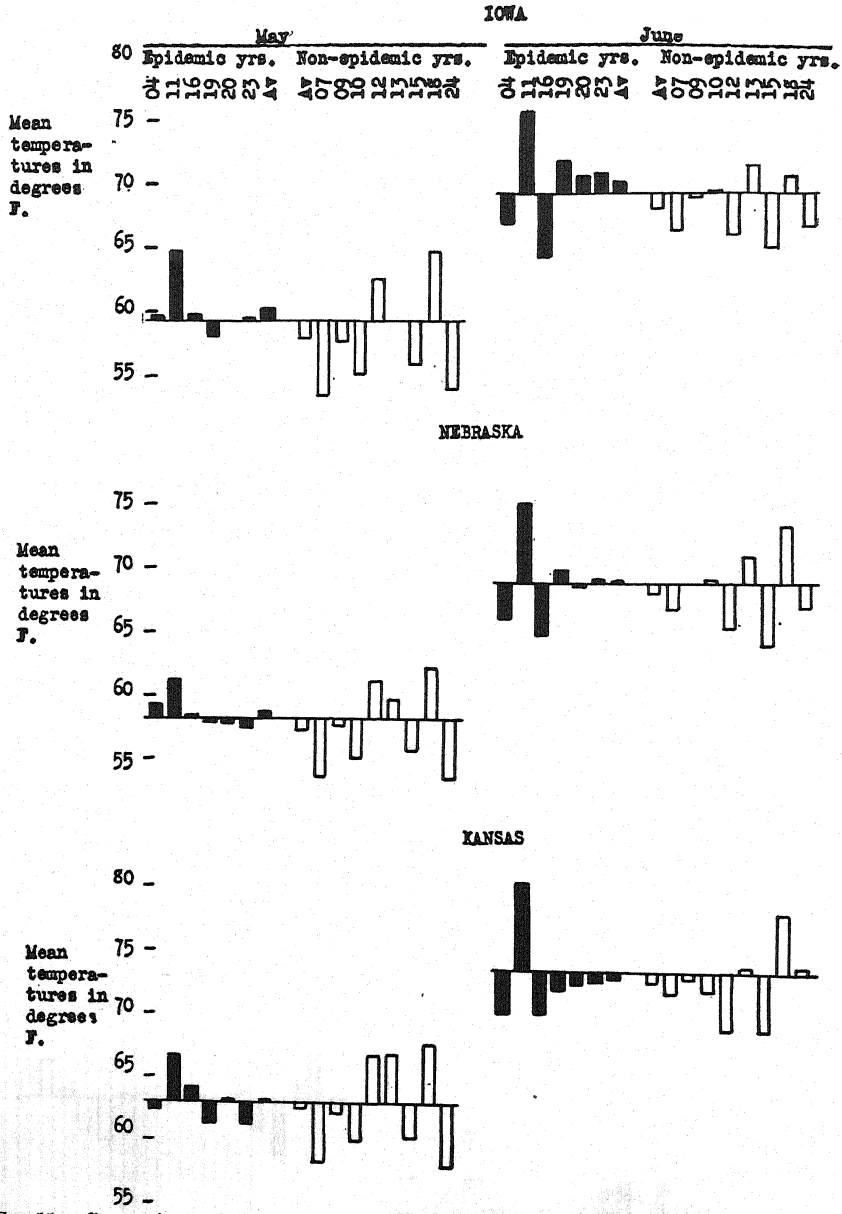


FIG. 11. Comparison of the departure from the normal temperature in Iowa, Nebraska and Kansas, which occurred during epidemic and non-epidemic years in May and June, from 1904 to 1925 inclusive.

in practically all late wheat fields near Morden, Man., Canada. This unusually severe epidemic developed in spite of the fact that there had been no rainfall in the vicinity of Morden for six weeks.

DISCUSSION AND CONCLUSIONS

Although the stem rusts of cereals are present every year throughout the Mississippi Valley, they do not develop uniformly in different parts of the valley nor in different seasons. In the Northern States barberries may become heavily infected, but they usually do not rust in Oklahoma or Texas. On the other hand, *Puccinia graminis tritici* and *P. graminis avenae* live throughout the winter in the uredinial stage in Texas and perhaps occasionally in Oklahoma, but there seems to be little or no overwintering in the Northern States. It is well known that the stem rusts of cereals are most

TABLE 19.—*Comparative meteorological data for May and June, the critical months for the development of stem rust in Missouri*

Year	May				June			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	63.1	5.03	12	13	70.5	6.42	11	12
1905	64.9	4.87	10	12	75.2	3.09	7	15
1906	66.3	2.16	7	15	72.7	4.95	10	15
1907	59.5	5.56	10	14	71.2	5.84	11	14
1908	66.1	6.92	12	12	72.3	6.30	11	13
1909	63.3	5.34	9	15	73.9	5.21	11	11
1910	60.1	6.62	13	11	70.4	3.92	8	16
1911	68.8	1.55	5	19	78.9	1.78	6	18
1912	67.6	3.86	7	18	69.5	4.78	9	14
1913	65.8	3.05	8	16	75.4	2.57	6	18
1914	66.6	1.47	5	17	79.3	2.37	5	18
1915	63.7	7.86	12	12	70.8	6.51	12	11
1916	65.6	5.61	11	15	70.0	6.99	10	14
1917	58.7	4.33	10	15	71.0	3.78	8	19
1918	68.3	5.49	10	16	77.2	3.15	7	17
1919	62.0	5.80	13	10	75.0	5.59	11	14
1920	63.8	4.85	12	9	72.7	2.35	6	18
1921	66.4	3.69	7	17	76.6	5.71	12	12
1922	67.2	3.17	10	14	76.5	1.66	5	19
1923	62.7	4.59	12	11	73.5	5.48	11	14
1924	58.6	4.67	10	13	72.5	7.39	14	12
1925	61.2	2.14	7	14	76.5	6.10	9	15
Averages	6.41	4.48	9.63	14.0	73.71	4.63	9.09	14.95

prevalent in the spring wheat area, where destructive epidemics frequently develop. The observations of the writer and of several other investigators indicate that these regional and seasonal differences in the development of epidemics are due at least partly to certain specific differences in climate.

Stakman and his coworkers have shown that barberries do not rust in the south because, in that region, the teliospores are not viable in the spring. They either germinate in the winter or are killed by the high temperature of the summer months. To the writer the latter possibility seems stronger, because in all of his experiments teliospores which had been subjected to high temperatures failed to germinate in the spring.

Attempts have been made to determine the particular factors which from year to year establish the border line between regions in which stem rusts overwinter and regions in which they do not overwinter. The evidence at

TABLE 20.—*Comparative meteorological data for May and June, the critical months for the development of stem rust in Iowa*

Year	May				June			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	59.6	3.78	8	13	67.1	3.45	7	13
1905	58.3	5.95	14	12	69.9	5.53	10	12
1906	60.8	3.54	11	13	67.9	3.92	8	15
1907	53.5	3.48	10	11	66.5	5.35	11	14
1908	59.4	8.34	15	9	67.1	5.66	13	12
1909	57.9	4.34	9	12	69.1	6.41	13	12
1910	55.4	3.41	10	15	69.5	1.99	7	18
1911	64.9	3.76	9	16	75.7	1.82	5	20
1912	62.7	3.33	10	14	66.2	2.74	7	15
1913	59.4	6.24	13	11	71.5	3.31	7	19
1914	62.2	3.31	10	14	72.2	5.57	13	12
1915	56.1	7.34	14	9	65.1	4.16	11	12
1916	59.9	4.93	12	13	64.5	3.71	10	13
1917	55.1	3.87	10	15	66.0	6.65	12	13
1918	64.9	6.87	13	13	70.8	5.29	11	16
1919	58.2	3.11	9	13	71.9	6.13	13	12
1920	59.4	3.26	8	14	70.7	3.56	9	16
1921	63.3	4.23	10	14	74.7	3.76	9	16
1922	63.4	3.53	12	13	72.2	1.82	6	19
1923	59.6	2.84	10	14	70.9	4.93	12	14
1924	54.1	1.71	9	13	66.8	8.10	14	11
1925	57.8	1.16	6	19	70.4	6.64	12	15
Averages	59.35	4.20	10.5	13.18	69.40	4.57	10	14.5

hand indicates that stem rust will overwinter readily only in regions in which alternate freezing and thawing are rare.

There are three possible ways for stem rusts to overwinter in the absence of their alternate host: urediniospores may remain viable throughout the winter and cause infection in the spring; dormant mycelium may persist in green leaves; or infection may take place repeatedly throughout the winter. Several workers have shown that, as a rule under field conditions, urediniospores lose their viability during periods of alternate freezing and thawing. Freezing temperatures do not seem to be so critical as alternate freezing and thawing, since in the Northern States urediniospores may remain viable throughout the winter months only to be killed in the spring at the time of alternate freezing and thawing. A reasonable explanation for this lies in the fact that urediniospores germinate at temperatures only

TABLE 21.—*Comparative meteorological data for May and June the critical months for the development of stem rust in Nebraska*

Year	May				June			
	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days	Mean temperature, degrees F.	Av. precipitation in inches	No. rainy days	No. clear days
1904	59.2	3.68	10	12	66.2	4.71	11	11
1905	56.2	5.73	12	9	68.4	5.04	11	10
1906	60.2	2.76	7	14	66.7	2.70	6	16
1907	53.7	2.88	8	11	67.0	4.03	9	15
1908	57.1	4.98	11	12	66.6	6.74	13	11
1909	57.6	3.23	9	12	69.0	4.26	10	12
1910	55.2	2.80	9	13	69.4	2.65	7	15
1911	61.2	2.27	6	14	75.2	1.76	4	20
1912	61.2	1.80	6	15	65.6	2.31	7	15
1913	59.8	3.60	9	13	71.1	2.73	7	18
1914	60.6	2.67	8	14	72.3	4.66	9	16
1915	55.8	5.25	12	12	64.2	5.81	13	10
1916	58.5	3.51	9	15	65.0	3.38	9	15
1917	53.2	4.85	11	12	67.0	3.24	8	19
1918	62.3	3.97	9	14	73.5	1.93	6	21
1919	57.9	2.54	7	16	70.0	4.94	10	16
1920	57.9	3.55	10	10	68.9	2.95	9	15
1921	60.7	3.62	9	12	73.0	2.35	7	17
1922	60.3	2.77	8	13	72.2	2.41	6	21
1923	57.5	4.99	11	13	69.3	4.70	10	13
1924	53.6	1.90	7	13	67.1	4.27	11	14
1925	58.7	2.06	6	16	70.9	4.46	11	15
Averages	58.11	3.43	8.82	12.95	69.02	3.54	8.82	15.22

slightly above freezing, while the minimum temperature for the penetration of the host is several degrees higher. Presumably the urediniospores germinate prematurely at temperatures too low for infection to take place. Peltier and others have shown that at low temperatures stem rust mycelium may remain dormant in wheat leaves for several weeks. North of Oklahoma, however, the chances for mycelium to live through the winter seem slight, because infection usually does not occur under field conditions later than October; in the cases studied the mycelium has always died in midwinter. In the district near Dallas, Texas, where detailed observations were made by Frolik, Butler, and the writer, in the winter of 1923–24, stem rust apparently was killed in January and February; while in the same winter, at San Antonio, Texas, the rust propagated itself throughout the winter. There were two striking differences between the weather conditions in these areas. In the vicinity of San Antonio, during the months of December, January, and February in that winter, there were four periods in which the mean temperatures ranged from 18° C. to 25° C. (65° F. to 78° F.), accompanied by sufficient rainfall for uredinial reproduction. At Dallas, on the other hand, such favorable conditions prevailed only once. Moreover, there was alternate freezing and thawing only four times at San Antonio, as contrasted with twenty times in the Dallas district. Here, alternate freezing and thawing seemed to check rust development by killing the host leaves bearing the old pustules as well as by causing viable urediniospores to germinate prematurely.

Of course, climate also affects the regional development of stem rust indirectly by determining the geographic limits of cereal crops. For example, *Puccinia graminis avenae* can not overwinter on grain north of Oklahoma because winter oats is not grown in the Northern States. Likewise, *Puccinia graminis secalis* can not overwinter in any great quantity south of Kansas because there is practically no rye grown in the south.

These in general appear to be the ways in which climate affects regional differences in the development of stem rusts. Another question of general interest is to what extent are seasonal fluctuations in rust development due to weather conditions?

This leads naturally to the question, can the development of destructive epidemics in the spring wheat area be correlated with certain weather conditions? There are two sources of inoculum which are known to contribute to the development of these epidemics: urediniospores or aeciospores which have been blown into the area from a distance, and aeciospores from barberries within the area. Assuming that these are the principal sources, it is evident that the weather might affect the development of epidemics in several ways: winter and spring weather conditions may affect overwinter-

ing or the accumulation of urediniospores in the south; the direction and velocity of winds may determine whether inoculum is blown into the spring wheat area; weather conditions in early spring may affect the severity and time of barberry infection; and, within the spring wheat area, weather conditions during the growing season may affect the rate of accumulation of inoculum, the rate of maturity of the host, and the amount of excessive transpiration caused by stem rust infection.

In southern Texas stem rust apparently overwinters more readily in some years than in others. But the problem of correlating this seasonal fluctuation with weather conditions is not a simple one. In the first place, it is difficult to determine the relative amount of overwintering from year to year because even in favorable years stem rust overwinters in only a few fields in a hundred. The amount of general primary infection in the spring can not be depended upon as an index to overwintering, as there is always the possibility of inoculum having been blown in from Mexico. The problem is complicated further by the fact that overwintering is so largely dependent on fall infection, which in turn is determined by factors such as oversummering of urediniospores, possible migration of inoculum from north to south in the fall, date of sowing of grain, and the time of emergence of volunteer grain in the fall. Because of the meagerness of our knowledge of these factors, no attempt has been made to correlate seasonal differences in overwintering with winter weather conditions.

In Texas stem rust accumulates most rapidly in April and May, while farther north in Oklahoma, Kansas, Missouri, Iowa, and Nebraska it develops principally in May and June. Temperature might be assumed to affect the rate of accumulation of inoculum in these states and thus indirectly affect the development of epidemics farther north. However, there is no indication of a correlation between mean monthly temperatures during these critical months in the Southern States and epidemics in the spring wheat area.

The diffused nature of primary infection in the spring wheat area suggests a distant source of inoculum. It has been shown by actual observation that spores are disseminated directly for several miles from barberries, and there is circumstantial evidence that spores are disseminated many miles from local epidemics near barberries. Stakman and his coworkers have shown that viable urediniospores are present in the air at high altitudes over heavily rusted fields. Observations on the movement of dust indicate that the wind is capable of carrying these spores long distances. Steady south winds frequently blow with sufficient velocity to carry spores from southern Texas to the spring wheat area in a few days. It is evident from the daily weather maps that each year for the past 25 years such air cur-

become infected in the south in all probability because teliospores are killed by the heat of the summer months. Alternate freezing and thawing seems to be the critical factor which determines the borderline between regions in which there is abundant overwintering in the uredinial stage and regions where stem rust does not overwinter extensively. In the spring wheat area there seems to be a tendency for epidemics to develop in warm growing seasons. Otherwise there is little or no evidence of a correlation between seasonal weather conditions and stem rust epidemics. There apparently are no other specific meteorological conditions or sets of conditions which usually accompany severe epidemics in the spring wheat area, and there is apparently no evidence of an association between weather conditions outside the spring wheat area and epidemics within the area. The problem is complicated by the presence of more than one possible source of inoculum and by the fact that conditions which favor the pathogene also may favor the development of the host. The development of severe epidemics on oats in years when stem rust is scarce on wheat, and *vice versa,* further illustrates the complexity of the problem. Similarly, the predominance of different physiologic forms of *P. graminis tritici* in different years must be considered. With so many interrelated factors involved, each epidemic appears to be a law unto itself. More precise information is needed, but the observer can not be cautioned too strongly against mistaking coincidences for causal relations.

SUMMARY

1. The weather affects the development of all phases in the life cycle of the stem rust organism.

2. Teliospores apparently lose their viability if kept for several months at high temperatures. It is suggested that this may explain the absence of rust on barberries in the Southern States.

3. Such factors as alternate freezing and thawing, alternate wetting and drying, light, and chemical stimuli did not have a measurable effect on the rest period of teliospores. Time seems to be the most important factor.

4. For the past five years at St. Paul, Minnesota, teliospores were shown to be viable several weeks before barberry leaves appeared in the spring.

5. After teliospores are matured, different lots of spore material, and even spores from different sori on the same straw, may require different lengths of time for germination. This probably facilitates barberry infection by insuring the germination of teliospores in several rainy periods.

6. Teliospores germinate in a wide range of H-ion concentrations, and apparently are not affected by light.

7. In nature teliospores usually lose their viability a few months after they mature but, if kept in cold storage, they may be preserved for nearly a year.

8. Field observations indicate that almost invariably teliospores can be found within a few rods of heavily rusted barberries.

9. The optimum temperature for the infection of *Berberis vulgaris* by *Puccinia graminis secalis* is 17°–18° C. Infection was sparing at 22°–23° C. and practically absent at 26° C.

10. Penetration of barberry leaves seems to be favored by low light intensity, while the development of aecial infection, after penetration, appeared to be favored by intense light.

11. There is some indication of a correlation between the mean temperature and rainfall in April and the earliness and severity of barberry infection in Minnesota.

12. Overwintering of urediniospores seems to be limited to seasons and regions in which temperature and rainfall favor the development of the uredinial stage at frequent intervals throughout the winter. Periods of alternate freezing and thawing are apparently a limiting factor.

13. In southern Texas weather conditions in summer seem to be more important as a limiting factor to the persistence of the uredinial stage than winter weather conditions.

14. In 1926 the predominance of stem rust on oats in northern Texas over that on wheat appeared to be associated with weather conditions during the previous year which were favorable for the development and persistence of volunteer oats.

15. The extent of the observable dissemination of urediniospores depends upon the amount of inoculum present. The rust from a single pustule usually develops through several generations before sufficient inoculum has accumulated to cause a noticeable infection at a distance of several rods.

16. When rust becomes plentiful throughout one or more fields of grain, it may spread, in observable amounts, for several miles to surrounding fields. There is circumstantial evidence that rust may be blown several hundred miles from a severely infected area.

17. Southerly winds have been shown to sweep up the Mississippi Valley, at the time stem rust is most plentiful in the south, with sufficient velocity to carry spores from Texas to the spring wheat area in less than three days. There is circumstantial evidence that conditions of this kind occurred from one to several times during the last ten days in May and the first ten days in June in every year for the past 25 years. But there is no apparent correlation between the prevalence of these winds and the occurrence of epidemics in the spring wheat area.

18. A comparison of the mean temperature in May, June, and July in epidemic years with that in non-epidemic years indicates that there is a correlation between warm growing seasons and destructive epidemics in the spring wheat area.

19. Differences in the total precipitation or the number of rainy days during May, June, and July do not seem to be associated with the development of epidemics. Field observations indicate that severe epidemics sometimes develop in parts of the Red River Valley in which rainfall is totally absent. It is suggested that heavy dew may account for this condition.

20. No evidence was obtained of any specific meteorological condition or set of conditions that always accompanies epidemics.

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A NEW VIRUS DISEASE EPIDEMIC ON ONIONS

I. E. Melhus, C. S. Reddy, W. J. Henderson and Edgar Vestal

This paper is a preliminary report of investigations of a new onion disease which occurred in epidemic form in the Pleasant Valley onion district of Iowa in May, 1928.

The sudden development of the disease in epidemic form on one of our most intensive crops, coupled with its unknown nature, warrants directing the attention of all pathologists to its presence, appearance, and destructiveness. Although comparatively little is known as yet about this disease, it is already reasonably certain that an additional cultural practice will be necessary in order to continue growing onions in areas where this disease becomes established. In all probability it will be necessary to index for the presence of the disease in mother bulbs and onion sets produced in diseased areas.

This virus disease did not come into existence this year. It was observed in June, 1927, in the same district, and specimens were collected. At that time the disease was not common and the growers considered it probable that the few affected plants either had been damaged by a hail storm or merely lacked sufficient vitality to produce normal growth. Isolations were made from a collection made at that time with only negative results, and results of inoculations with the juice from crushed tissue on other onion tops also were negative.

The seriousness of the disease was first recognized in May, 1928, when 95 per cent of the mother bulbs planted to produce seed were found to be diseased in some fields. A little later the disease was observed to be prevalent in the principal acreage planted with onion sets. Onions are grown from sets as well as seed for commercial purposes in this district. Here the infection ranged from 15 to 50 per cent, although in a few fields as many as 95 per cent of the plants were infected. The financial loss cannot be determined accurately, but 25 per cent is a conservative estimate of the loss induced this season in the Pleasant Valley section.

Later in the season commercial onions grown from seed became diseased, but the plants showed the symptoms at about the same stage of growth as those grown from sets. Where seed was planted thickly to produce sets, the disease appeared later in the growing season, when the plants had developed five leaves. The symptoms were less evident than on plants grown from mother bulbs and sets.

Fields in outlying sections where the growers used their own sets were much freer from this disease. In fact two fields were located where neither the seed crop nor the commercial onion crop grown from sets showed a single infected onion. In other fields in outlying sections where the sets had been purchased from growers in the Valley the percentage of infection was as high as observed in the more intensive onion district.

SYMPTOMS AND COMMON NAME

The symptoms of this new virus disease are very striking and conspicuous. In advanced cases the plants are severely stunted and yellow, the lopped-over leaves superficially suggesting a wilt. In the field on

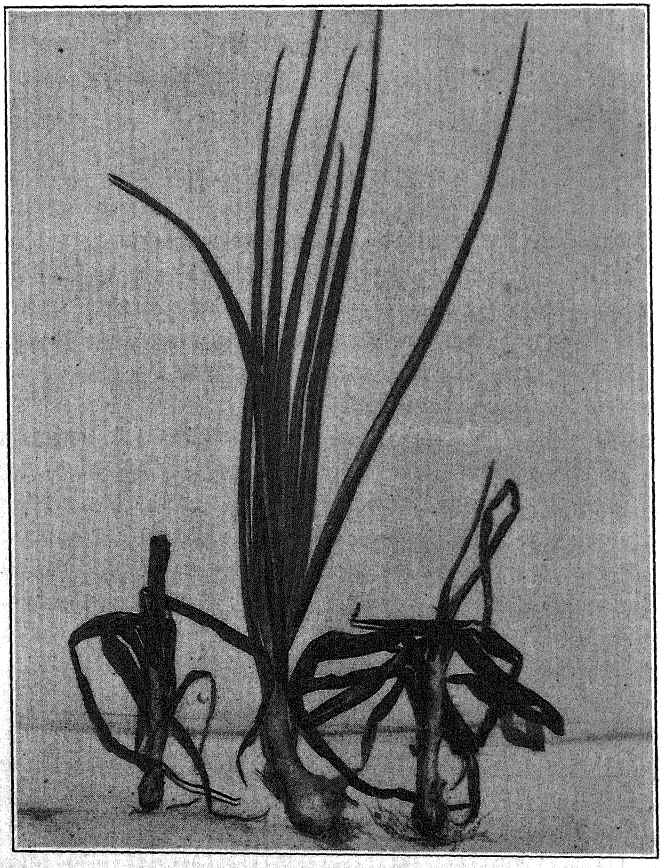


FIG. 1. Yellow dwarf disease on two Yellow Bottleneck onions and a healthy onion of the same variety between them, grown from sets at Pleasant Valley, Iowa, 1928.

plants from sets the symptoms usually first appear on the fifth leaf, but this may be owing to masking on account of temperature, because when sets are planted later than normally in warm soils in the greenhouse the disease symptoms often occur on the earlier formed leaves.

On the leaves are yellow streaks which sometimes extend the full length of the leaf. In other cases the yellow streaks are much shorter. Such leaves as a rule are chlorotic throughout, are crinkled and flat, lose their turgidity and lop over with the tips touching the ground. In other cases only a part of the leaf may show symptoms. The flower stalk does not react to the disease like the leaves, but remains turgid and round although it is often chlorotic, bent and twisted, and from 6 to 10 inches shorter than normal. Marked stunting occurs on all diseased plants, but particularly on those grown from sets and mother bulbs. The stunting is least on the onion sets grown from seed. As stunting and yellowing are two of the conspicuous symptoms, the common name suggested for this disease is "yellow dwarf of onions" (Fig. 1).

SPREAD OF THE DISEASE

From numerous observations and experiments it is known that the disease is carried in the onion sets and mother bulbs, but not in the seed. Seed grown in the Pleasant Valley district in 1927 and planted in three places in Iowa, one in Wisconsin, and one in Minnesota, in 1928, produced crops free from the disease. On the other hand, wherever onion sets were distributed in a like manner, the disease occurred, showing that the vegetative stage of the host may carry the disease over winter. In greenhouse experiments at Ames, Iowa, the disease developed whenever sets or mother bulbs grown in the Pleasant Valley district were used. It was at first thought that no symptoms appeared during the year that infection occurred; but later in the season the disease occurred on onions grown from seed. Since the disease is not carried on the seed, it must have been transmitted to the young plants during the current year.

CAUSE OF THE DISEASE

The character of the disease symptoms and the repeated failures to find an organism consistently associated with the disease suggested a virus.

The following experiments tend to substantiate this supposition. Onion plants infected with the yellow dwarf disease were crushed by means of a small food chopper. A small quantity of distilled water was added to this crushed material and the mixture ground in a mortar. After stirring for a few minutes, the diseased juice was filtered and the filtrate used in experiments.

The onion sets used in the six inoculation trials recorded were obtained from William Hartz, of Pleasant Valley, Iowa. In his field planted from this lot of sets about 10 per cent of the plants were infected. It was the healthiest lot of sets of the Yellow Bottleneck variety available when the inoculations were undertaken. This sort is one that has been improved in this district and the one preferred by all the growers. The sets were inoculated by injecting into them about 1/10 cc. of the juice from diseased onions. Each time that 100 onion sets were inoculated and planted, 100 uninoculated sets from the same lot were planted as checks. The results of these inoculation experiments are recorded in table 1.

TABLE 1.—*Inoculation experiments on units of 100 Yellow Bottleneck sets in which infection was determined by subsequent growth from the sets growing in the field, Pleasant Valley, Iowa, 1928*

Trial no.	Date planted	No. days between planting and final reading	Inoculated sets; no. diseased	Check sets; no. diseased
1	June 22	24	59	16
2	" 30	17	22	7
3	" 30	17	20	7
4	" 30	18	30	6
5	" 30	18	29	6
6	July 6	12	26	3

Table 1 shows that the highest percentage of infection occurred in trial No. 1; the checks showed 16 per cent and the inoculated lot 59 per cent. Probably the method of inoculation was the most important variable in these trials. In the first trial the inoculum was injected deeper into the bulbs than in the other five trials. Without exception a higher percentage of diseased plants developed in the inoculated sets than in the uninoculated. The average was 31 per cent infected plants in the 600 inoculated sets and 7.5 per cent in the checks. These results tend to support the supposition that the cause of the disease is a virus and that it can be transmitted artificially by injections of juice from diseased onion plants into other onion sets. More than four times as many yellow dwarf plants developed from the 600 onion sets inoculated than from 600 similar onion sets not inoculated. The symptoms of the yellow dwarf disease manifested themselves in 9–12 days when the plants were growing rapidly. If the plants are not growing rapidly it may require a longer time, and if the growth is very slow no signs of the disease will develop.

Although the experimental work has not progressed far enough to justify definite statements relative to control of this disease, nevertheless these suggestions may be of value to other workers. The most practical way of combatting this disease is to use sets known to be disease free. However, when it is necessary to obtain seed stocks from an infested district, the distinctiveness of the symptoms coupled with the promptness with which they appear under good growing conditions suggests that stocks of sets and mother bulbs may be readily indexed before planting for the presence or absence of the disease.

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ABSTRACTS OF PAPERS PRESENTED AT THE TWENTIETH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, NEW YORK, N. Y., DECEMBER 28, 1928, TO JANUARY 1, 1929

Relation of the removal of smut balls to the control of stinking smut in winter wheat. R. S. KIRBY.

Three years' tests have shown that when winter wheat containing 1–10 per cent of smut balls is treated with copper carbonate dust and is planted by grain drills, approximately an equal percentage of smut results in the succeeding crop. When the same wheat is planted by hand without rupturing the smut balls, practically no stinking smut occurs in the succeeding crop.

This led to a study of the efficiency of cleaning machinery in removing smut balls. A combination cleaning and treating machine of the screen-and-air-draft type, with a capacity of 60–75 bushels of wheat an hour, removed 91.5 per cent of the smut balls. A cockle disc cleaner removed 83.3 per cent while cleaning 100–150 bushels an hour. A receiving separator of the screen-and-strong-air-draft type, cleaning 100–150 bushels an hour, removed 100 per cent of balls when less than 3 per cent was present, but let a few through at over 3 per cent. Running the seed through both the disc machine and receiving separator removed all smut balls in every lot containing less than 10 per cent. These figures are based on the treatment of over 31,000 bushels of wheat with these machines.

The influence of environment during maturation upon predisposition to seedling blight in wheat and corn strains. JAMES G. DICKSON, P. E. HOPPE, J. R. HOLBERT, and GEORGE JANSSEN.

The past three years' tests have shown a marked relation between the environment during the period of maturation and the subsequent growth responses of the seedling as well as the complete seasonal development and yield of the crop. High-yielding, resistant strains of spring wheat became susceptible and poor producers when the seed was grown one season under the hot, midsummer conditions of central Illinois, whereas the seed of the same strains matured at Madison, Wis., resulted in resistant plants and yields characteristic of the strains.

Similar results have been obtained in late-maturing inbred lines of corn when grown at Madison under unfavorable fall conditions. This so-called immature corn even in highly resistant inbred lines has been completely susceptible to seedling blight and produces plants and ears of markedly inferior quality, unlike those from the original line or from plants from well-matured ears of the same line. This does not seem to be a disturbance of the genetic composition of the line but rather the expression of the characters. There is little difference in endosperm composition, but marked changes in the embryo. This influence of the maturation environment is of special importance in comparing strains or varieties matured under different conditions. (Cooperative investigations between the Wisconsin Agricultural Experiment Station, Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and Funk Brothers Seed Company.)

Inheritance of resistance to seedling blight of corn caused by Gibberella saubinetii. P. E. HOPPE.

In studying mode of inheritance of resistance to seedling blight of corn caused by *Gibberella saubinetii*, selfed strains of corn have exhibited wide range of relative resis-

tance when inoculated and grown at 16° C. Their consistent behavior at this temperature indicates high degree of homozygosity for the genetic factors involved in resistance.

F_1 hybrids from a resistant × susceptible cross were as resistant as the resistant parent. Hybrid vigor does not necessarily mask susceptibility, as shown by a susceptible × susceptible cross which was as susceptible as either parent.

Analyses of 27 F_3 families gave evidence of transgressive segregation, as families were isolated more susceptible and more resistant than parent strains. One F_3 family, practically immune, maintained its high degree of resistance in the F_4, while results in other families indicated that the F_3 families had been very heterozygous.

Environmental factors as well as degree of maturity of seed greatly affect expression of resistance. It is necessary therefore to test all genetic generations simultaneously and under the same environment before one can expect an accurate analysis of the manner of inheritance of resistance to this disease. Material is available for such a study to be conducted during the winter of 1928–29. (Cooperative investigations between Wisconsin Agricultural Experiment Station and Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.)

Susceptibility of barley varieties to Fusarial head blight in Minnesota. J. J. CHRISTENSEN, H. A. RODENHISER, and CHIH TU.

Scab was unusually prevalent on barley in southern Minnesota in 1928. At University Farm, 132 varieties and selections, grown in duplicate series, were subjected to an artificial epidemic produced by several species of *Fusarium*. Some varieties were extremely susceptible, some highly resistant, still others intermediate. The percentage of infected heads ranged from a trace to 100 per cent. A correlation coefficient of $+.50 \pm .04$ was obtained between the behavior in the two series. The heads of some of the most susceptible varieties were completely ruined, *viz.*: Meloy, C. I. 1176; Club Mariout, C. I. 261; Flynn, C. I. 1311; and many others. Some of the resistant varieties grown in Minnesota are: Improved Manchuria, Minn. 184 (17 per cent); Svansota, Minn. 440 (7 per cent); Peatland, Minn. 452 (4 per cent). In Glabron, Minn. 445, and Velvet, Minn. 447, two recently developed smooth-awned varieties, 49 and 68 per cent of the heads respectively were infected. Fusarial head blight may become an important factor in barley production if susceptible varieties are developed and supplant the more resistant ones. There is no apparent correlation between varietal resistance to scab and to spot blotch.

Ethyl mercury chloride as a seed grain disinfectant. W. H. TISDALE and W. N. CANNON.

The E. I. duPont de Nemours and Company has investigated many materials in an effort to develop a dust with a greater range of effectiveness than is possessed by the copper, formaldehyde, and certain of the mercury dusts on the market. Chemists of the company suggested ethyl mercury chloride as a possibility. In toxicity tests, this product proved highly effective against certain bacteria and fungi. Different dust combinations, each including a number of strengths of the toxin ranging from 0.5 to 4 per cent, were prepared and tested on infested seeds. They proved highly effective in the control of the diseases but in some cases the seeds were injured. Combinations and strengths have since been prepared which cause no injury to the seeds of the small grains, but give excellent disease control. Very satisfactory results have been obtained in the control of bunt of wheat, covered smut and the stripe of barley, loose smut of Tennessee Winter barley, the smuts of oats, and covered kernel smut of sorghum. For control of these diseases a thorough dusting of the seed with a 1.5 per cent product has been found sufficient. Tests are under way on other seeds. (Bayer-Semesan Company.)

Experiments with liquid and dust seed disinfectants for controlling covered smut of barley and stinking smut of wheat, 1926–1928. R. W. LEUKEL.

Covered smut of barley was eliminated without seed injury by soaking seed one hour in 0.5 per cent solution of Uspulun, Semesan, or Bayer Compound or in 0.25 per cent solution of Germisan, Corona 620, or Tillantin "C" with 2.5–7.5 per cent smut from untreated seed.

Among 40 dusts tested, Abavit B, Trockenbeize Tillantin, and Wa Wa reduced covered smut to a slight trace without seed injury and also reduced loose smut from 0.7 per cent to a slight trace. Untreated seed produced from 3.6 to 12.2 per cent heads affected with covered smut.

For controlling stinking smut of wheat, 44 dusts were tested. Perfect control was obtained without seed injury with Abavit B, Trockenbeize Tillantin, Wa Wa, Vitrioline, S. F. A. 225 and 225 V, Dupont 68, and Ceresan. Commercial control was obtained with copper carbonate in all cases except one in 1927, when copper carbonate containing 18 per cent copper reduced stinking smut from 25 to 8 per cent. Heavy rains after sowing doubtless were responsible for this failure.

Copper carbonate does not deteriorate with age but some other dusts do. Extremes of soil reaction did not affect the fungicidal efficiency of the dusts used. Very dry soil conditions during germination and emergence reduced fungicidal action of the dusts.

Experiments on stripe disease of barley and its control. R. W. LEUKEL, J. G. DICKSON, and A. G. JOHNSON.

Over 50 dust disinfectants of American and foreign manufacture were tested for the control of stripe disease in several varieties of barley. Ceresan, Trockenbeize Tillantin, and Wa Wa practically eliminated stripe in every trial. Abavit B, Tutan, Semesan, Mercury "C," S. F. A. 225 V, and several experimental dusts were usually effective in stripe control but not consistently so. Uspulun, Germisan, and Semesan used in solution were effective as heretofore. The age of the seed showed no relation to the effectiveness of the fungicides used.

The dusts did not greatly increase either germination or stand. Yield, however, was increased by the effective treatments, both with liquids and dusts.

Relatively dry soil (less than 20 per cent saturation) during the period of emergence seemed to favor stripe development as compared with very wet soil. Stripe developed most abundantly at or below 15° C., some occurred at 20° C., a trace at 24° C. and none at 28° C. Changing the soil temperature from 12° C. to 28° C., or vice versa, after the first-leaf stage did not affect the percentage of stripe. (Cooperative investigations between Wisconsin Agricultural Experiment Station and Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Preliminary report on a bacterial disease of corn. A. G. JOHNSON, LILLIAN CASH, and W. A. GARDNER.

In June a disease of corn, now known to be caused by bacteria, was reported from Baldwin County, Alabama, by County Agent S. H. Gibbons. Later, what seems to be the same disease was found to limited extent on Arlington Farm, Va.

The disease manifested itself as a stalk rot of upper part of stalk and as a leaf blight. The stalk rot occurred usually at or just above where ears are produced, causing dark brown soft rot of internodal pith with no infection in lower part of such stalks. This rotting usually killed the tops before or just after tassel emergence, producing the effect of marked dwarfing of plants. Leaf blight occurred on middle and upper leaves. Lesions were usually stripes of various widths and lengths, sometimes coalescing and

killing greater portions of some leaf blades. The younger lesions were watersoaked; older lesions were dry in centers, light tan with sepia margins. Later, attacked leaves became greatly shredded. Microscopic examination of young lesions showed invaded tissues teeming with bacteria.

Numerous isolations have been made and some cultures have proved pathogenic on both leaves and stalks.

Both the disease manifestations and the organism apparently differ distinctly from those of the bacterial stalk rot previously described by Rosen. (Cooperative investigations between Alabama Agricultural Experiment Station and Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Another bacterial leaf spot of sorghum. CHARLOTTE ELLIOTT.

This disease, observed in Texas, Oklahoma, and Kansas, is characterized by dark reddish brown streaks on the leaves of *Holcus sorghum*. The youngest lesions are narrow, watersoaked streaks. Narrow margins or blotches of reddish brown soon appear in these streaks, and in a few days the red color is continuous throughout the lesions. At intervals these red streaks broaden out into elongated oval spots with tan centers and narrow red margins. Exudate is abundant and dries down to white or cream-colored scales. Isolations have given pure cultures of a yellow organism, the pathogenicity of which has been proved. The organism is apparently undescribed.

Net-necrosis of Irish potato tubers. G. H. GILBERT.

Net-necrosis of potatoes, consisting in the appearance of a network of brown strands in the vascular region of the tuber, is shown to involve the sieve-tubes, companion cells, and adjacent cells of the phloem parenchyma which together constitute the brown strands that are evident when the skin and outer cortex of affected tubers are removed. Earliest indications of necrotic changes are the occurrence of dead and discolored protoplasmic contents of sieve-tubes and companion-cells. Later larger areas occur, as a result of the merging of smaller spots. Necrotic areas are frequently separated from surrounding normal tissue by the formation of cork cambium walls.

Microchemical tests show that changes in necrotic walls involve accumulations of suberin, lignin, and pectic substances and result in strong resistance to cell-wall solvents.

Field experiments have further confirmed the hypothesis that net-necrosis of the tuber is correlated with leafroll and particularly that it is a first-season symptom following initial leafroll infection. Characteristic necrosis was produced under controlled conditions following the transfer of aphids from leafroll to healthy plants in cages, while tubers from control plants remained free from the disease.

Apical leafroll of potato. E. S. SCHULTZ and REINER BONDE.

Apical leafroll, a new potato disease, is related to the leafroll group of virus diseases. Infected plants slightly dwarfed manifest roll of the upper leaves similar to primary leafroll, and to the roll of the apical leaves frequently associated with rhizoctonia and similar diseases. However, apical leafroll is distinguished from primary leafroll by persisting only on the upper leaves in succeeding generations. The tubers, reduced in size but normal in shape and number, are frequently but not invariably attached to short stolons resembling the rather clustered tuber set of witches'-broom plants. Apical leafroll plants are distinguished from those affected with witches' broom by taller, more vigorous, and fewer shoots which form larger and fewer tubers.

Observations on apical leafroll during four seasons have disclosed that it is perpetuated through the tubers and that it can be transmitted to healthy potatoes by tuber grafts. Experience with Green Mountain, Rose No. 4, Irish Cobbler, and Bliss Triumph varieties has shown that they are susceptible to this disease and that, although Green Mountain and Rose No. 4 manifest the foliage symptoms earlier in the season than the other varieties mentioned, the most conspicuous symptoms appear too late in the growing season to insure effective control.

Calcium arsenate as a fungicide. H. H. WHETZEL, S. E. A. MCCALLAN, and T. C. LOH.

In spraying and dusting tests during the summer of 1928 on the control of *Alternaria panax* on ginseng, it was found that Pyrox entirely prevented infection by this pathogene. Bordeaux mixture (4–4–50) and copper lime dust (20–80) gave little control, merely retarding somewhat the final destruction of the stems and foliage. Laboratory tests with these fungicides using spores of *Alternaria panax* indicate that the effective fungicidal agent in the Pyrox, as far as this fungus is concerned, is the arsenate of lime rather than the copper. Arsenate of lead which is also present in Pyrox showed but little toxic action on the spores of this fungus. A 1 per cent mixture of calcium arsenate in talc proved to be more toxic to the spores of this fungus than a 4–4–50 bordeaux mixture. Since it is well known that Alternaria pathogenes are in general not easily controlled with copper protectants, the evidence in this case points to the possibility of more effective control of diseases such as early blight of potatoes by the use of calcium arsenate alone or in combination with copper protectants.

A preliminary report on forced-air ventilation for the control of Cladosporium leaf mold of greenhouse tomatoes. A. G. NEWHALL and J. D. WILSON.

All attempts to control Cladosporium leaf mold of greenhouse tomatoes by spraying or dusting with either copper or sulfur fungicides have failed.

The conidia will germinate over a temperature range from 6° to 29° C. They require a relative humidity above 96 per cent. Humidity is, therefore, of the greatest importance in determining the rate of spread of the disease.

Transpiration is found to be the greatest factor responsible for high humidities in greenhouses. As many as 400 gallons of water an hour have been computed to be given off by an acre of tomatoes under normal conditions in June. The problem of controlling leaf mold is therefore one of ridding the house of this excess water vapor and keeping the leaf surfaces comparatively dry.

Apparatus is being developed for studying the effects of different rates of air change under controlled humidities on spore germination and leaf mold development. A study has been made of the evaporating power of the air in a number of greenhouses equipped with forced-air ventilation systems of different types. Brief descriptions will be given of some of the installations now on trial in Ohio greenhouses with notes on costs, methods of operation, and yields.

Effects of some environmental factors on the root-knot nematode. G. H. GODFREY and HELENE T. MORITA.

Heterodera (*Caconema*) *radicicola* lives for months under favorable conditions. Egg masses may hatch 100 per cent under best conditions. Larvae are killed at 43° C. in 10 minutes and at 53° instantly. Egg masses are killed at 48° C. in 10 minutes and at 58° instantly. Complete dryness kills larvae in 3 minutes and egg masses in 2 hours. Drying at 70 per cent relative humidity kills larvae in 10 minutes and egg masses in 4 hours. Free eggs are killed more quickly than those in egg masses. Egg

masses buried within roots survive for 48 hours in complete dryness, for 5 days at 60 per cent, for 7 days at 70 per cent, and indefinitely at 90 to 99 per cent. Tests with sunlight and with ultra-violet lamp (moisture and temperature favorable) show that ultra-violet light kills nematodes. Sunlight killed larvae in 25 minutes, egg masses in 8 hours, free eggs in 1½ hours. Combined sunlight and drying killed larvae in 2 minutes, egg masses in 30 minutes, and eggs within roots in 8 hours. X-rays did not kill larvae or eggs in 35 minutes. In greenhouse tests with soils 10 inches deep, the organism was completely killed by 4 months' drying with stirring; when the soil was not stirred, so that the bottom was still moist and the humidity high in interspaces, there was only partial killing. Field results indicate practical application of this definite information.

A Fusarium disease of beans.—L. L. HARTER.

In August, 1928, a disease was observed for the first time in the Sacramento Valley of California on several varieties of field beans. Isolations made from the fibrovascular bundles from various parts of the stem gave in every case a species of *Fusarium* belonging apparently to the Elegans group. The fungus traverses the fibrovascular bundles throughout almost the entire length of the stem and extends into the branches and into the petioles of the leaves. The fibrovascular bundles are conspicuously discolored. The plants were not observed to wilt like most plants invaded by a vascular parasite. They were somewhat dwarfed. In the early stage the color of the leaves changed to pale green, later to a bright yellow. The leaves finally dried up and dropped off. Diseased plants could be recognized in the field at some distance by bright yellow foliage.

Inoculations have been made and typical symptoms of the disease produced. Specimens of diseased plants have not been collected outside of the Sacramento Valley.

Further studies on the control of stem rot of sweet potatoes. R. F. POOLE.

The stem rot, wilt, yellows, or root rot disease of sweet potatoes caused by *Fusarium batatatis* was controlled on farms in different parts of North Carolina by treating stems and roots with bordeaux mixture as described in 1927. A 20–20–50 bordeaux mixture gave the best control without injury. A 5–5–50 mixture gave some control, but less than the stronger mixtures. Slight injury was caused by a 50–50–50 mixture. A 25 per cent monohydrated copper-lime dust gave equally good control as the liquid treatments without causing injury in any test. These treatments were not effective when diseased plants were used. They did not increase or decrease production when the yields from treated plants were compared with healthy, untreated plants. Disease was controlled equally well in Porto Rico, Nancy Hall, and Yellow Jersey varieties.

Further studies on watermelon wilt in Iowa. D. R. PORTER and I. E. MELHUS.

In 1928 all local commercial varieties of watermelons tested proved to be susceptible, although individual plants of some appeared to be resistant. Two edible foreign varieties proved resistant. The variety Conqueror again failed to show appreciable resistance, but the F_3 generation of some Kleckley-Conqueror crosses appeared to possess considerable resistance. Seven citron strains were tested and six of them proved almost immune. The F_3 generation of Kleckley-citron (variety Kafir) crosses proved to be 86 per cent resistant, but few of the melons were edible.

One hundred per cent wilt occurred after 22 days when seed of the variety Tom Watson was planted in infested soil kept between 22° and 25° C. In soil kept between 16° and 19° C., none of the Tom Watson plants wilted after 22 days and only 51 per cent after 49 days. In soil kept between 22° and 25° C., none of the stock citron plants wilted after 22 days and only 21 per cent after 30 days.

Chemical injury to watermelons in transit—results of shipping tests. W. W. GILBERT and F. C. MEIER.

In 1925 a report was made on experiments to determine whether chemicals commonly hauled in freight cars are able to injure watermelons subsequently loaded in such cars, and if so, the extent and character of this injury. In the course of two years' tests under actual shipping conditions, the results indicate that certain chemicals, or commercial fertilizers containing one or more of them, are dangerous when present on the walls and floor of the car. By using ample dry bedding, injury to melons was avoided.

When scant dry bedding was used, serious injury (discoloration, pitting, softening) was caused by common salt, sodium nitrate, acid phosphate, ammonium sulfate, potassium sulfate, and potassium muriate. Injury was more severe than results of earlier stationary tests had indicated would be the case. Greater injury was caused if the bedding was damp when the melons were loaded. In none of the tests was serious injury caused by hydrated lime or pulverized road lime, though slight superficial damage resulted when wet bedding was used. The results point to the necessity of cleaning cars thoroughly before loading, and using ample bedding, that is, the equivalent of two to three 75-lb. bales of straw.

A fruit rot of honey dew melons due to a species of Phytophthora. CHARLES DRECHSLER.

From the decayed tissue of a large lesion appearing externally as an extensive, somewhat depressed, water-soaked region on a honey dew melon, *Cucumis melo* var. *inodorus*, originating presumably in California or Colorado, was isolated in Washington, D. C., Sept. 15, 1926, a species of *Phytophthora* producing on irrigated lima bean agar prominently papillate zoosporangia with an average length of 57 μ and an average diameter of 30 μ. The fungus, on inoculation into healthy fruits, proved an efficient parasite in causing a decay similar to that present in the original specimen. Too great a specificity should not be attributed to the pathogenicity thus evidenced, since the large majority of fungi referable to the genus *Phytophthora* appear to be capable of inducing identical pathological effects with greater or less rapidity. Among the more rapidly destructive of congeneric parasites are to be included, for example, *P. cactorum* (Cohn & Leb.) Schroet., *P. citrophthora* (Sm. & Sm.) Leon., *P. melongenae* Saw., *P. parasitica* Dastur, and *P. terrestris* Sherb.

Breeding for resistance to cucumber mosaic disease. E. E. CLAYTON.

On Long Island, cucumber mosaic is regular in its occurrence, and attempts to control it by weed eradication have failed completely. This is a first report on an attempt during four years to select, out of the standard pickling and slicing varieties, strains that are mosaic resistant. Inbreeding did not reduce plant vigor but did bring out many abnormalities, particularly in fruits, *i.e.*, albino, spiny, and misshapen types. Off-type lines were discarded and the desirable ones tested for resistance to downy mildew (*Pseudoperonospora cubensis*) and bacterial wilt (*Bacillus tracheiphilus*) as well as mosaic. All lines have proved equally susceptible to mildew. Numerous lines showed extreme susceptibility to wilt, and a few showed moderate resistance. Lines that show distinct mosaic resistance were secured, as well as many that were extremely susceptible. The development of mosaic-resistant varieties similar in type to varieties now in commercial use appears entirely practicable.

Reaction of Chinese cucumbers to mosaic. R. H. PORTER.

In the summer of 1928 an occasional plant of the cucumber variety Chinese Long (collected at Nanking, China, by the writer in 1926) following inoculation with mosaic

virus from the variety Chicago Special developed a faint mottling which was frequently difficult to detect. Attempts to transfer the virus from such plants to susceptible American varieties were successful to the extent that 80–100 per cent of the plants inoculated developed mosaic. Later it was found that some plants of the Chinese cucumber developed a decided crinkling of the terminal leaves without showing any yellowing or mottling. Transfers from such plants to American varieties proved the presence of the mosaic virus. In no case has "white pickle" or stunting of any of the Chinese plants been observed either in the field or greenhouse, such as commonly occurs on American sorts. The percentage of plants in this Chinese variety which develop either mottling or crinkling after inoculation is low, probably not over 15 per cent. The incubation period required for development of symptoms also averages longer than in American varieties. The above-described symptoms disappear within 5–15 days, even when the plants continue to grow vigorously.

The yellow dwarf of onions. I. E. MELHUS and W. J. HENDERSON.

A new disease of onions, first noted in 1927, became epidemic in the Pleasant Valley district, in Iowa, in 1928. It is characterized by stunting of bulbs, and crinkling, yellowing, and drooping of leaves. The prevalence of the disease varies from a trace to 95 per cent of the plants infected. It appeared first in a low place where the water had run over the field and was most severe in areas that had been flooded. Evidence to date indicates that the disease is not seed borne. It is carried in the vegetative stage, sets, and mother bulbs. Inoculation trials with juice of diseased onions show that it is a transmissible virus disease. The incubation period is about 12 days. In trials involving 700 sets, 34.3 per cent became infected as a result of injecting the juice of diseased onions into buds of the sets before planting. Inoculation trials on tomato, cucumber, tobacco, and squash have not been successful. The name "yellow dwarf" is proposed.

Toxicity of mercury and copper compounds in relation to their use for seed treatment and spraying. E. E. CLAYTON.

Many recommendations for spraying and seed treatment involve the use of copper and mercury compounds in concentrations likely to be toxic to plants. These effects are complicated and often not at once evident. Thus lima bean seed dusted with a mercury compound may germinate well but the plants produced are smaller and yield less than those from untreated seed. Again, healthy gladiolus corms soaked 7 hours in 2.0 per cent Semesan were never killed, but they failed to produce as large plants or flowers as the untreated. Species vary in susceptibility to injury, and closely related forms may react quite differently. Thus, while lima beans were mercury-sensitive, string beans were not. Comparing the seed and the growing plant, tomato seed was sensitive to injury from mercury disinfectants and tomato plants resistant to injury from mercury sprays. Cucumber and cantaloupe seed were resistant to injury from mercury and copper disinfectants but the plants were sensitive to injury from the sprays. More fundamental knowledge of the behavior of copper and mercury as plant poisons will make possible safer and more effective use of control measures.

Progress report on studies of fireblight of apple. P. W. MILLER.

The results of three years' study of the epidemiology of fireblight of apple show that, under Wisconsin conditions, meteoric water is an important agency in disseminating the primary and the secondary inoculum. The rôle of insects in disseminating fireblight appears to be less important than was earlier believed and needs to be re-evaluated.

Abundant infection of young shoots and unopened blossom buds on potted apple and pear trees grown in the greenhouse under conditions designed to prevent injury by insects and other agencies has been induced at will by spraying them with a pure suspension of *B. amylovorus* in sterile distilled water and placing them in a moist chamber at suitable temperatures. Numerous cases of stomatal penetration have been found in histological studies of tissues taken from (a) inoculated young apple leaves, (b) the sepals of inoculated unopened apple flowers, and (c) the inside of the receptacle cups of apple and pear flowers that were open when inoculated.

These results seem considerably to enhance the possibility of using the excision method as one part of a successful control program. Strong potentialities appear to lie in the use of chemical treatments designed to inactivate such primary inoculum as escapes the excision process.

Rust diseases of the apple. H. E. THOMAS and W. D. MILLS.

Three rust diseases are destructive to the apple in eastern New York State: apple rust caused by *Gymnosporangium juniperi-virginianae* Schw., hawthorne rust caused by *G. globosum* Farl., and quince rust caused by *G. germinale* (Schw.) Kern. Quince rust has been found on the fruit of five varieties of apples, causing heavy losses. The symptoms vary widely from one variety to another. Hawthorne rust occurs on the foliage of at least 13 varieties. In a few instances the infection is severe. Apple rust has been unusually abundant on both fruit and foliage in 1927 and 1928. The peridial cells of the fungi seem to offer the most distinct diagnostic characters.

Studies of the fungicidal action of certain dusts and sprays in the control of apple scab. J. M. HAMILTON.

The investigation was conducted in the greenhouse on the leaves of potted apple plants under controlled conditions in order to relate fungicidal applications with the experimental production of disease as desired. Leaves treated with sulphur dusts or lime-sulphur spray before infection were efficiently protected until resistant. Sprays containing lime-sulphur gave good control when applied 72 hours after inoculation and in such treatments were consistently more effective than the sulphur dusts, colloidal sulphur sprays, copper fungicides, oils, and proprietary mercury compounds tested.

Temperature appeared to influence the development of host and parasite and progress of disease more than it affected the fungicidal action of sulphur.

No significant increase in fungicidal efficiency of sulphur followed the addition of potassium permanganate to sulphur as an oxidizing agent.

After various fungicidal treatments potted plants were washed and inoculated. Bordeaux mixture and sprays containing lime-sulphur were found to be decidedly more adhesive than certain sulphur dusts, colloidal sulphur sprays, oils and other combinations. The addition of emulsified oil and certain other materials increased the effectiveness of certain fungicides.

Laboratory studies corroborated the greenhouse studies of the sulphur fungicides with respect to the relation of temperature and the addition of potassium permanganate.

Third progress report of studies of fall applications of fungicides in relation to apple scab control. G. W. KEITT and E. E. WILSON.

In 1927, in continuation of studies which have been reported (Phytopath 17: 45 and 18: 146), scabbed apple leaves were sprayed with various fungicides after harvest and before leaf-fall. Representative samples of control and treated leaves were overwintered and studied for the development of perithecia of *Venturia inaequalis*. Many of the com-

pounds tested showed some value in suppressing the development of the perithecia. Calcium arsenite (probably chiefly ortho) gave very promising results. In several tests, calcium arsenite, in concentrations varying from 0.25 to 1 per cent, alone or mixed with other materials, reduced the number of perithecia to less than one-tenth the number which developed in the controls. Certain of these calcium arsenite preparations have caused serious host injury, while others have been applied with a power sprayer to orchard trees without evidence of injury in the following season. Because of the high toxicity of calcium arsenite, it is not yet recommended for commercial trial, and caution in its experimental use is advised.

The effect of sprays on the weight of cherry fruit. E. E. WILSON and G. W. KEITT.

Studies of the weight of cherry fruits from trees which had been sprayed comparatively with bordeaux mixture and lime-sulphur, respectively, have been conducted for the past five years. The results have varied considerably from year to year and orchard to orchard. In general, fruits from trees sprayed with lime-sulphur were somewhat heavier than those from trees sprayed with bordeaux mixture. The increased weights of lime-sulphur over bordeaux sprayed fruits from Montmorency trees were as follows for the following years: 1924, 15 per cent; 1925, 13 per cent; 1926, 9 per cent; 1927, 16 per cent; 1928 (average of four orchards) 8 per cent. The increased weights of lime-sulphur over bordeaux sprayed fruits in four orchards in 1928 were as follows for the following varieties: Early Richmond, 1 per cent, 4 per cent, 6 per cent, 8 per cent; Montmorency, 2 per cent, 6 per cent, 11 per cent, 13 per cent. This increase in weight in 1928 appeared to be due to increase in the fleshy part of the fruit, as no significant difference could be found in the weight of pits of fruits from trees receiving the two different treatments.

The influence of the form and proportion of lime and copper sulphate on the suspension of bordeaux mixture. F. J. SCHNEIDERHAN.

Approximately 1,000 tests of bordeaux mixture, prepared according to 30 different formulas and from different forms of lime and copper sulphate, were made to determine suspension percentages. Stone lime, hydrated (finishing) lime, chemical hydrated lime, crystalline copper sulphate, powdered copper sulphate, and dehydrated copper sulphate were used, both in stock solutions and by combination in dry form. The highest ratio between suspended bordeaux and the total volume of liquid was found when chemical hydrated lime and finely granulated copper sulphate were used according to the formula 2–3½–50. A close correlation between fineness of lime particles, its chemical composition (especially the CaO content) and suspension ratios exists. Chemical hydrated lime containing 72.7 per cent CaO; 98.74 per cent passing through a 325-mesh sieve and with a volume of 3.04 cc. per gram, when used in combination with finely granulated copper sulphate resulted in a maximum suspension of 98.8 per cent after one hour, 97.6 per cent after two hours and 95.2 per cent after three hours. The same materials used in 2–4–50, 3–4–50, 3–5–50, and 1–1–50 bordeaux gave suspension ratios nearly as high as the 2–3½–50 and considerably higher than any formula of bordeaux prepared from slaked stone lime and copper sulphate in stock solutions. Excellent fungicidal properties of "Instant" bordeaux prepared from powdered materials have been demonstrated in the control of apple blotch (*Phyllosticta solitaria*) during two years.

Dusts composed of lime-sulphur and sulphur. H. C. YOUNG.

Four sulphur dust combinations with finely ground dry lime sulphur were tested for control of apple scab. These were 85–15 and 90–10 sulphur-dry lime sulphur, 85–10–5 sulphur-dry lime sulphur-bentonite, and 85–10–5 sulphur-dry lime sulphur-hydrated

lime. The 85–15 combination gave excellent control during a season that was exceptionally favorable for scab development. This dust proved superior to any others used except the oxidized sulphur dusts.

Chemistry of the toxic factor of sulphur. R. C. WILLIAMS and H. C. YOUNG.

Studies on the chemistry of the toxic factor of sulphur have been continued. Ordinary ground roll and flowers of sulphur were found to yield varying amounts of the toxic factor depending upon reaction, oxidation, temperature, and moisture. Sulphurs treated with such oxidizing agents as potassium permanganate, manganese dioxide, and arsenious acid were found to be extremely toxic. All known acids associated with sulphur were analyzed and tested for their toxicity and the only ones found toxic were the polythionic (tetra and penta) acids. All sulphurs freed from these acids were nontoxic to *Venturia inaequalis*. Several oxidized sulphur dusts were tried in the field and found effective in the control of apple scab. It was found further that the toxic factor was destroyed by alkalis and strong acids.

Adhesiveness of sulphur mixtures. H. C. YOUNG and L. E. TISDALE.

It was found in this work that materials such as bentonite, kaolin, hydrated lime, gypsum, glue, or any other type of so-called stickers and fluffers do not enhance the adhesiveness of 300-mesh ground roll sulphur. Particulate sulphur stuck better than coarse particles. Sulphur found on leaves after heavy rains was in a very fine state, about 600 mesh. Different types of rains influenced the adhesiveness of sulphur, a heavy downpour washing off less sulphur than the same amount of water falling more slowly and over a longer period. It was found that when a heavy coating of sulphur dust was applied to leaves or slides less would remain after rains than when smaller amounts were applied. Apparatus and methods for slide determination of sulphur adhesiveness are described.

Some raspberry mosaic symptoms. C. W. BENNETT.

Further experimental results in the transmission of raspberry mosaics indicate that symptoms of mottling may be caused by a larger number of viruses than has heretofore been recognized. The terms yellow, mild, and red raspberry mosaic may be used to indicate groups of related symptoms, with no clear line of separation, however, between the more severe forms of mild mosaic and the lighter forms of red raspberry mosaic. Yellow mosaic seems to be a very definite type distinguishable from the other mosaics by color of foliage, time of incubation, and rate of dispersion of the virus in the plant. Viruses of this type seem to be much more virulent from some sources than from others. Other mosaics differ chiefly in severity of symptoms. There is a wide range of effect produced, varying from a very light mottling in the milder forms to a severe leaf distortion and cane-tip necrosis which has been described in red raspberry mosaic. Frequently, viruses of these types are combined with yellow mosaic, from which they can be separated by virtue of their more rapid movement. Plants affected by the milder forms remain susceptible to infection by more severe types; thus some of the severe types of mosaic may be caused by a combination of two or more viruses.

Decay of citrus fruits in transit. HARRY R. FULTON and JOHN J. BOWMAN.

The rots here considered are caused by *Penicillium digitatum, P. italicum, Phomopsis (Diaporthe) citri,* and *Diplodia natalensis.* Data are given showing the prevalence of these rots on arrival at destination, and the degree to which various amounts of decay depress the market price. This price depression measures financial loss, and sets limits

to the profitable employment of control measures. Relative effectiveness of various control measures is discussed, based on experiments extending over many years. These show (1) that Diplodia stem-end rot may be reduced 50 per cent or more by early spring removal of dead wood in which the fungus sporulates, (2) that Phomopsis stem-end rot may be reduced 50 per cent or more by a one application of bordeaux mixture to the very young fruit, (3) that both types of stem-end rot can be reduced 90 per cent or more by prompt removal of the stem "buttons" after picking, (4) that chemical treatments in packing houses can reduce stem-end rots and blue mold rots very greatly, (5) that precooling and refrigeration are important protective measures during transit and marketing, (6) that promptness and care in handling at all stages are essential in successfully combatting rots of citrus fruits.

Further studies on Exoascaceae. A. J. MIX.

Attempts to isolate *Exoascus deformans* and *E. mirabilis* from fragments of diseased tissue are successful only when asci form on these fragments in culture and ascospores are shot on to the agar.

Conidia of *E. deformans* formed in culture are uninucleate. Nuclear division occurs in the budding conidium and a daughter nucleus passes into the bud. Copulating conidia and long germ tubes with binucleate cells as reported by Wieben for *Taphrina epiphylla* and *T. klebahni* have been observed. Cells from culture, previously called resting cells, are believed to be ascogenous cells. They show paired nuclei, fusing nuclei, or a large single nucleus. Binucleate hyphae with plate septa and structures believed to be imperfect asci have also been found. Apparently growth in culture must be initiated by the haploid (saprophytic) stage, but, in established cultures, cells belonging to the diploid generation occur.

Exoascaceae thus far cultured include: *Exoascus deformans, E. mirabilis,* and *E. mirabilis* var. *tortilis* from *Prunus angustifolia, E. pruni* from *P. domestica, Exoascus sp.* from *Alnus incana* and *A. rugosa, E. communis* from *Prunus americana,* and *Taphrina coerulescens* from *Quercus rubra.* The two last-named forms do not agree in cultural characters with cultures described by Martin as of *Taphrina communis* from *Prunus americana,* and *T. coerulescens* from *Quercus rubra* and *Q. nigra.*

A disease of tobacco seedlings caused by Septomyxa affinis (Sherb.) Wr. W. B. TISDALE.

Each year since the initiation of tobacco investigations in Florida a disease of tobacco seedlings has been observed in several seed beds on new land. The disease usually occurs in portions of the beds partially shaded by trees, but in 1928 under very humid conditions entire beds were invaded. Infected areas of the beds are conspicuous because of the yellowed and stunted condition of the plants. The disease is characterized by more or less irregular, olivaceous blotches on the upper surface of the leaves and blotches or streaks of similar characters on the petioles and stems. Under humid conditions the affected parts develop a soft rot, resulting in a ragged appearance of the leaves and the damping off of the petioles and stems. Plants artificially inoculated with spores of the fungus and kept under humid conditions in the greenhouse develop severe damping off within five days. Slightly infected plants recover when transplanted to the field.

A similar disease of tobacco seedlings has been reported from Connecticut, Kentucky, and Ohio and has been attributed to a species of *Fusarium.* A study of the morphological characters of the fungus shows that it closely resembles *Fusarium affine* Sherb., which according to Wollenweber's new combination becomes *Septomyxa affinis* (Sherb.) Wr.

Studies in the physiology and cytology of Ustilago zeae and Sorosporium reilianum. W. F. HANNA.

The sporidia of *U. zeae* are haploid. Corn plants inoculated with monosporidial cultures never produce galls, but those inoculated with mixtures of certain pairs of monosporidial cultures produce galls which bear mature chlamydospores. On germination, some chlamydospores give rise to two pairs of sporidia of opposite sex; the sporidia from other chlamydospores may belong to four sexual groups. Segregation of factors may occur at both divisions of the spore nucleus. Sporidial conjugation has not been observed. The germ tube from a haploid sporidium is able to penetrate the epidermis of a young corn leaf. The diploid mycelium is initiated by a fusion of the germ tubes from two sporidia of opposite sex.

S. reilianum is heterothallic. Sporidia of four sexual groups may be produced from a single chlamydospore. Young corn plants develop galls only when inoculated with a combination of two monosporidial cultures of opposite sex.

Single monosporidial cultures of *U. zeae* and *S. reilianum* were mixed together and injected into young corn plants, but none of the inoculated plants developed galls.

Pathogenicity of multisporidial and monosporidial cultures of Ustilago zeae (Beckm.) Ung. A. H. EDDINS.

Five multisporidial cultures of *Ustilago zeae* isolated from one smut gall differed in pathogenicity when seedling plants of inbred lines of corn were inoculated hypodermically with conidial suspensions of these cultures.

Five monosporidial cultures derived by a dilution method from multisporidial cultures failed to produce smut galls on plants of five inbred lines of corn. When the inoculum of each of the monosporidial cultures was mixed with each of the other cultures, it was found that certain of the paired combinations produced chlamydospores in host tissue while other combinations did not. These results indicate that a culture originating from a single sporidium constitutes a sexual strain of the fungus. The five cultures were found to represent four such strains and were designated A, B, C, and D. Chlamydospores were produced in host tissue when A was paired with C, but not when paired with B and D. Chlamydospores were produced also when B was paired with C and D. No chlamydospores were produced when C was paired with D.

A fungous disease of conifers related to the snow cover. J. H. FAULL.

A disease of conifers, especially devastating to the spruces other than black, occurs in the snow-laden parts of Northeastern America, and it may occur in the West. It is due to a *Phacidium* that answers to *P. infestans*. The form as found everywhere on balsam may be the same as Weir's variety *abietis* on western *Abies*. *Phacidium* blight attacks *Abies, Picea, Thuja,* and *Pinus* sporadically in the native forests, but the spruces, and particularly white spruce, to an alarming extent when the blight gets a foothold in nurseries and plantations. The disease develops and spreads by mycelium under the snow cover in early spring, but not during the summer. Primary infection from ascospores probably takes place in the autumn.

Perfect control has been attained by spraying in the late fall with lime sulphur, and by suitable sanitation measures. Such control has almost completely eliminated the disease from certain affected nurseries and plantations. Without control in these areas, the success of reforestation with white spruce at least is gravely doubtful.

The development of the spores of Plasmodiophora vascularum. MELVILLE T. COOK.

Plasmodiophora vascularum is a plasmodium which lives in the tracheary tissue of the sugar-cane, but which does not cause a hypertrophy. The plasmodium produces a

number of uninuclear masses which appear to be slightly amoeboid. They increase in size and produce thick cell walls. The spores form short germ tubes. The organism travels through the tracheary tissue to a considerable height in the plant. It is distributed in the seed-cuttings and possibly in other ways.

A diplanetic species of Phytophthora causing pink rot of potato tubers. CHARLES DRECHSLER.

A species of *Phytophthora* with prominently papillate zoosporangia averaging approximately 54 μ in length and 37 μ in diameter was isolated from potato tubers affected with watery decay originating near Ft. Gibson, Oklahoma, June, 1923, and from tubers showing mealy pink rot originating near Louisville, Kentucky, August, 1923. On artificial inoculation it causes pink rot evidenced internally by purplish discoloration of eyes and in death of buds; while cut affected tissues on exposure to air rapidly turn pink and later blackish. Though similar pathological effects are produced also on inoculation not only with *Phytophthora erythroseptica* Pethy., but also with such congeneric types as *P. cactorum* (Cohn & Leb.) Schroet., *P. cinnamomi* Rands, *P. citrophthora* (Sm. & Sm.) Leon., *P. cryptogea* Pethy. & Laff., *P. melongenae* Saw., *P. parasitica* Dastur, *P. parasitica* var. *rhei* Godfrey, and *P. terrestris* Sherb., the fungus exhibits considerable specific distinctiveness in energetic diplanetism. Primary zoospores after rounding up yield secondary ones by direct delivery of the protoplast through an evacuation tube 3 μ wide and 1–7 μ long; or less directly by the production terminally on a pedicel 1.5 μ wide and 5–25 μ long of a definitely papillate, ovoid, 1-spored germ-sporangium delimited by a basal septum. The latter manner of development, exceedingly frequent with zoospores imprisoned within primary sporangia through partial or complete frustration of normal discharge, results often in a novel reproductive arrangement. Diplanetism in some measure occurs elsewhere in the genus, as, for example, in a form referable apparently to *P. erythroseptica* and isolated earlier from Idaho potatoes by G. K. K. Link.

A Stemphylium leaf spot of tomatoes. GEORGE F. WEBER.

An undescribed leaf disease of tomatoes has been noted in Florida during late spring and early summer for the past five seasons. Primary infection takes place on the leaves near the soil surface in the seed bed or in the field. Secondary infection rapidly involves the entire foliage, the disease being most conspicuous on the topmost leaves. The spots produced by the fungus are sunken, grayish in the center, and surrounded by a dark band. They are small, usually 1–4 mm. in diameter and may be round, oval, or irregular. Repeated isolation from spotted leaves consistently yielded the organism, which grows well and sporulates readily on potato-dextrose agar. The organism remained viable at 5° C., but showed little or no growth below 10° C. Its optimum temperature ranged between 23° and 26° C., with the maximum temperature for growth below 35° C. Optimum temperature for sporulation was 32° C. The hyphae, conidiophores and conidia are fuliginous and septate, and the conidia are muriform. The conidia germinate readily in 2–6 hours in water. Inoculation experiments have proved its parasitism on several tomato varieties, as well as peppers, ground cherries, eggplants, and *Solanum floridanum*. Bordeaux mixture 4–4–50 applied weekly after plants are up has proved to be the best fungicide for the control of this disease.

Local symptoms of mosaic in the leaves of some Nicotiana species. F. O. HOLMES.

At least five species of *Nicotiana* show necrotic local lesions where tobacco mosaic virus successfully enters their leaf tissues. These species are *Nicotiana rustica, N. acuminata, N. langsdorffii, N. sanderae,* and *N. glutinosa. Nicotiana glutinosa* shows

pronounced localization of symptoms on the leaf inoculated. The young leaves near the growing point usually show no evidence of the disease. This extreme localization is correlated with rapidity of development of the necrotic lesions, which appear about 30 hours after inoculation and are fully formed in 4 to 5 days. The lesions are useful for measuring virus concentrations, for studying methods of inoculation, and for the isolation of pure lines of virus. *Nicotiana tabacum* does not show necrotic lesions, but gives evidence of the effect of the virus at the site of inoculation by changes in leaf color and amount of starch formation.

Are blackfire and angular leaf spot of tobacco identical? W. D. VALLEAU.

Attempts to control blackfire of dark tobacco by means of sanitary measures designed to exclude *Bacterium angulatum* from the plant bed, while often giving favorable results in controlling true angular leaf spot, have evidently failed to control blackfire, as it occurs following topping and suckering. Results of field, isolation, and inoculation studies and pot-culture work raise the question as to whether angular leaf spot and blackfire are not distinct diseases, the former bacterial and the latter physiological, and associated with certain nutritional and seasonal conditions. In pot cultures with a soil low in available nitrogen, spots similar to those of blackfire have developed following rapid growth which resulted from heavy applications of a nitrogen fertilizer, whereas the spots have been prevented by a single further application. Field-plot results in Kentucky and elsewhere suggest a relation between potash applications and control. Heavy applications of stable manure appear to control blackfire. Isolations from recently developed blackfire spots have failed to yield *Bact. angulatum,* while older spots sometimes appear to be contaminated with this organism. The blackfire or physiological type of spot is characterized by concentric rings, while typical angular leaf spots, as they occur in the field or result from inoculations, are not zonate.

Progress in the control of black shank of tobacco through disease resistance. W. B. TISDALE.

The original selections in the development of a strain of Big Cuba tobacco resistant to black shank (*Phytophthora nicotianae* Breda de Haan) were made in 1922. From one of these selections a highly resistant strain of merchantable quality has been developed. Attempts to isolate resistant strains of other varieties of cigar-wrapper tobacco by selection have been less successful. Since Big Cuba tobacco is considered of relatively mediocre quality, crosses have been made in an effort to combine its resistance with the superior quality of leaf of certain susceptible varieties.

Certain F_5 selections of the progeny resulting from a cross between resistant Big Cuba and Cuban have shown 98 per cent resistance and a quality of leaf superior to Big Cuba. Crosses between resistant Big Cuba and Connecticut Round Tip have proved less successful. Certain F_3 selections of progeny resulting from crosses between F_3 individuals of the Big Cuba-Cuban and Big Cuba-Round Tip progenies were practically immune to black shank and a leaf quality superior to that of any type tested.

The black-shank of tobacco in Porto Rico. J. A. B. NOLLA.

During the past two years a disease of tobacco known in Porto Rico as "pata-prieta," and probably the same as black-shank in the United States, has been studied. Plants of all ages are affected, the disease being especially destructive in seedbeds. The pathogene has been found to be *Phytophthora nicotiana* Breda de Haan. The Porto Rican fungus is supposed to be a slightly different strain from that causing a

similar malady in Florida. The pathogene seems able to produce a diseased condition in *Ricinus communis*, eggplant, pepper and tomato seedlings, and in potato plants.

The disease attacks both the cigar-binder and cigar-filler tobacco varieties. A non-commercial mammoth variety and the cigar-binder (Connecticut Round Tip) have proved most susceptible. A Porto Rican binder variety has shown high resistance.

Moisture is a dominant factor in outbreaks of damping-off caused by this pathogene; irrigation water is an important transportation agent.

As control measures are recommended: removal of diseased individuals, selection of seedlings, dipping healthy seedlings from diseased beds in bordeaux mixture (4–4–50 or 5–5–50) and the development of resistant varieties.

Verticillium wilt of cotton. C. D. SHERBAKOFF.

So far as known the first report on the occurrence of cotton wilt caused by *Verticillium alboatrum* (Reinke & Berth.) was made by the author in 1928 (Plant Disease Reporter, Supplement 61: 283 and 284. July 1, 1928). The additional information obtained since then shows: first, that the disease is present not only in Lake County, Tenn., but also in other places along both banks of the Mississippi River on a similar type of soil—various grades of "gumbo"; and, second, that the main field symptoms of the disease that distinguish it from the wilt caused by *Fusarium vasinfectum* are complete shedding of the leaves, and noticeable shedding of young bolls before the withering of the tips and branches, and an absence of any discoloration of cambium, with frequent development of new branches at the base of the plant. In case of the wilt caused by *Fusarium*, no conspicuous leaf or boll shedding or production of new branches at the base was observed, while in case of Verticillium wilt a distinct discoloration of the cambium was always noticed. The discoloration of fibrovascular bundles is always present in both wilts though it seems to be more evenly distributed in the case of the Verticillium infection.

A nematode root lesion of pineapple and other crops. G. H. GODFREY.

One factor contributing to root failure in pineapples in Hawaii is a root lesion produced by a species of *Tylenchus*. Lesions on young roots are irregular, longitudinal, argus brown spots, from one to several millimeters long, extending through cortical parenchyma cells but not into the stele. The nematodes multiply and migrate in the cortex, destroying the cells, until finally the root is killed. Branch feeding roots are often greatly reduced in number. The nematodes are sluggish, and lightly infested roots grow away from them and continue to function. When soil infestation is sufficiently heavy so that numbers of nematodes invade root tips, severe injury results and further growth of the root is prevented. Nematode root knot checks forward growth, making conditions ideal for penetration and multiplication of the *Tylenchus*, which then rapidly breaks down the tissues. Presence of the *Tylenchus* in a gall is always indicated by a brownish color. The two nematodes together make a destructive combination. Other plants subject to attack are tomato (slightly), soybean, cowpea (very heavily), and many weeds. In contrast with *Heterodera* (*Caconema*) *radicicola* the *Tylenchus* is resistant to drying. Its numbers are greatly reduced, however, in moist soil fallow, probably by starvation.

Further studies on the dry-rot canker of sugar beets. C. M. TOMPKINS.

Additional observations and experiments have been made during the past two years on the dry-rot canker (*Corticium vagum*) of sugar beets, first described by Richards of the Utah Agricultural Experiment Station in 1921. In late summer of 1927, the disease

was found for the first time in southern Idaho, and also in the San Fernando Valley, Calif., where it assumed epidemic proportions. Marked differences exist between the type of canker produced on beets in California and that found in Utah and Idaho. Pure-culture work indicates that there are at least two distinct strains of the fungus which induce similar host responses. Apparently these strains become pathogenic only in the presence of high soil temperatures in midsummer, which would explain the lack of seedling sickness in soils known to harbor the fungus. Field inoculation experiments show that the mycelium can penetrate the periderm without previous mechanical or other injury to the beet root. The number of plants affected tends to increase each succeeding year in contaminated soils, indicating constant extension of the organism within the soil. Since carefully conducted experiments in Utah have proved that crop rotation measures are ineffective, development of resistant strains of sugar-beet seed probably offers the most promising method of control.

Delayed thinning as a means of controlling damping-off of sugar beets. S. B. NUCKOLS and C. M. TOMPKINS.

Damping-off of sugar-beet seedlings constitutes one of the main limiting factors in securing good beet stands. In a field or locality, the number of infected seedlings fluctuates with the soil and seasonal conditions. Infection varies from 1 to 50 per cent in the cotyledon stage, although in extreme cases it may be 90 or 100 per cent. Results of three years of experimental work in Utah and western Washington on a wide range of acid and alkaline soils, indicate that good stands may be obtained by delaying thinning until the seedlings have six leaves, even when fields have been 50 per cent infected in the cotyledon stage. During this interval of about 20 days, sick seedlings are eliminated by natural means. When thinning is done in the six-leaf stage there is usually less than 4 per cent infection, which greatly minimizes the danger of leaving sick seedlings. Since determination can easily be made as to whether a six-leaf seedling is healthy or diseased, based upon the condition of the petioles, the possibility of leaving sick seedlings is practically eliminated. Healthy six-leaf seedlings and both healthy and sick two-leaf and four-leaf seedlings have straight leaf petioles, whereas diseased seedlings having six or more leaves have bowed leaf petioles. Employment of this method which involves primarily delay in thinning, elimination of seedlings with bowed leaf petioles, and permitting the largest healthy beet to remain as a single offers a practical means of control and entails no extra operating expense.

Vacuolar changes in diseased cells. J. DUFRENOY.

The bulk of the plant cell is normally made up of one or a few large vacuoles, originating from the numerous smaller vacuoles of the meristematic cell swelling and fusing together as the cell ages. That evolution in the vacuolar system may be reversible is evident in healthy glandular cells and in many diseased cells where the breaking down of large vacuoles into a number of smaller ones seems to be a frequent occurrence. After staining portions of leaf epidermis in the living condition with neutral red, or staining with haematoxylin or acid fuschin after proper killing, clusters of small vacuoles around invading hyphae of *Puccinia* may be seen in *Arisaema*, and similarly in cells around hyphae of *Puccinia asphodeli* in cells of *Asphodelus subalpinus*. Clusters of small vacuoles, surrounded by deeply staining cytoplasm, are also conspicuous in cells of discolored areas of mosaic-infected plants.

In any case, the breaking down of large vacuoles into smaller, spherical or thread-like vacuoles is suggestive of a local proteolytic process.

Studies on the influence of environment on infection by certain bacterial plant parasites. A. J. RIKER.

In certain diseases where the pathogen penetrates the host through natural openings, the moisture condition of the plant previous to inoculation has been found to be very important in relation to the amount of infection secured. Experiments have been conducted upon the various host plants of the following pathogens: *Bact. lachrymans* Smith and Bryan, *Bact. pisi* (Sack.) E. F. S., and *B. tabacum* Wolf and Foster. In each case it was found that plants placed a day in a moist chamber previous to inoculation and then placed again in a moist chamber developed a great many more lesions than plants which were given the moisture treatment only after inoculation.

Temperature was also found to be important in the development of infection. The optimum, as measured by the size of lesions on the respective host plants, was found to be approximately 24° C. for *Bact. lachrymans*, 28° for *B. tabacum*, 12° for *Bact. holci* Ken., 28° for *Bact. coronafaciens* Elliott, and 28° for *Apl. michiganense*, E. F. S.

The maturity of inoculated leaves appears to influence both the number of infections and the size of the subsequent lesions. It has been observed in certain cases that the newly expanded leaves commonly developed more lesions than older leaves.

Seed infection by Bacterium phaseoli. W. J. ZAUMEYER.

Bacterium phaseoli causes a systemic invasion of the bean plant under certain conditions, as shown by Burkholder and others. Recent studies have shown that at times, the organism may pass into the developing seed through the vascular system of the plant, without the production of any visible macroscopic symptoms. From dorsal suture infection of the pod, the bacteria invade the funiculus, passing through the raphe into the seed coat. Entrance may also be made through the micropyle of the seed when the bacteria invade the pod cavity. They may enter the pod cavity either through the stomata of the pod or by breaking through the vascular tissue of the pod sutures. In the seed coats the bacteria traverse the intercellular spaces which are very large and afford an easy passage for the rapid migration of the pathogen throughout these tissues. Later they are found surrounding the cotyledons, but penetration into these structures before germination has never been observed. At the time of germination with the imbibition of water the seed swells rapidly and many of the epidermal cells of the cotyledons are pulled apart with the formation of natural rifts. It is through these tissue tears that the bacteria enter and penetrate into the cotyledons. This later results in the systemic invasion of the developing seedling.

Studies on bacterial pustule of soy bean. S. G. LEHMAN.

The presence of a pustular outgrowth has been emphasized as the chief diagnostic character by which the lesions of bacterial pustule may be distinguished from those of other bacterial diseases of the soy bean. In numerous instances the writer has isolated the organism of bacterial pustule (*Bacterium phaseoli* var. *sojense*) from lesions devoid of any vestige of pustular outgrowth. Other organisms isolated from the same lesions failed to infect soy beans in the inoculation tests.

Bacterial pustule disease was produced when the source of inoculum was diseased leaves kept air dry in the laboratory for nine months or diseased leaves which had been subjected to weathering and attendant decay for three months.

Seed treatment with a large number of dusts and disinfecting solutions have failed to control the disease. Tests in which a large number of varieties of soy bean were repeatedly inoculated artificially, both in the greenhouse and the field, showed the variety Columbia to be most highly resistant.

Bacterial spot of radish and turnip. HAROLD E. WHITE and M. W. GARDNER.

A bacterial spot disease of radish and turnip was found in Indiana in 1928. Abundant spotting occurred on the leaf blades, petioles, stems, and seed pods of radish. Bacteria forming similar yellow colonies were isolated from the two host species, and seedlings of both became infected in the greenhouse as a result of cross inoculations. The petiole lesions on radish were very destructive. Evidence of transmission on radish seed was obtained. In greenhouse inoculation tests the organism also infected cabbage, brussels sprouts, cauliflower, mustard, and tomato.

Migration of Bacterium tabacum through the leaf tissues of Nicotiana tabacum. J. BEN HILL.

The migration of *Bacterium tabacum* Wolf and Foster, the causal organism of wild fire, has been studied in the leaf tissues of the host, *Nicotiana tabacum.* It has been found that the bacteria migrate as zoogloeae from the region of the inoculation puncture, and penetrate the intercellular spaces of the uninjured host tissues. The form of the zoogloeae is determined largely by the shape of the intercellular space penetrated. This is in accord with the observation of the zoogloeae of other bacterial phytopathogens which have heretofore been studied. In the early stages of migration the zoogloeae pass along through the intercellular spaces, often almost surrounding some of the mesophyll cells but frequently causing no apparent injury to the protoplast. Vessels when injured seem often to be penetrated by the bacteria. The rate of migration of the bacteria, as expressed by the growth of the zoogloeae, is extremely slow when compared with that of the crown-gall organism (*Bacterium tumefaciens*) and is much slower than that of the fire-blight organism (*Bacillus amylovorus*). Few zoogloeae were observed in microscopic preparations made of the tissues less than 48 hours after inoculation. Preparations made three days after inoculation showed many zoogloeae.

The chromosome number in crowngall and cancer tissues. MICHAEL LEVINE.

This report includes the results of my studies of the chromosome numbers in crown-gall cells of *Beta vulgaris* and *Nicotiana glutinosa*, in human epithelioma, and in mouse carcinoma No. 180. The cells in the reduction divisions in the beet show 9 chromosomes, while in the vegetative divisions only 18 chromosomes are found. In the crowngall tissue some cells show 18 chromosomes while many other cells contain 36 chromosomes. In a small percentage of cells approximately 72 chromosomes are to be seen.

In *N. glutinosa* the somatic divisions show 24 (diploid) chromosomes. This number is commonly found in dividing cells of crowngall tissue on this plant, although the tetraploid number (48) frequently occurs. Cells with still larger numbers of chromosomes have been observed, although I am not certain as to the exact count.

In human cancer a complete count of the chromosome number in giant cells is difficult. In a number of cases I found over 200 chromosomes. In spermatogenesis of the white mouse, 20 chromosomes may be counted in the primary and secondary spermatocytes. In transplanted mouse tumor cells, 40 chromosomes appear frequently, while cells with larger numbers of chromosomes are common.

The polyploid cells seem to originate through the failure of the cell to divide after nuclear division.

Studies on the bacteriological differentiation of the crowngall and hairyroot types of bacteria. W. H. WRIGHT, A. J. RIKER, H. E. SAGEN, and W. M. BANFIELD.

A comparison of the crowngall (*Bact. tumefaciens* Smith and Town.) type of organism with the type which causes hairyroot has been made from the standpoint of

both their morphological and physiological characters. A number of strains (isolations from different sources) were employed which were purified by special means; and, in the case of crowngall organisms, cultures of single-cell origin were used as a basis of comparison. The crowngall and hairyroot organisms have been found to differ in regard to the following characters: (1) motility; (2) growth, absence of growth, and growth characters in nine different synthetic media; (3) carbohydrate fermentation, including differences in true acidity (pH) and titrateable acidity, of xylose, arabinose, glucose, galactose, mannose, lactose, maltose, rhamnose, and erythrotol; (4) action on litmus milk; (5) absorption of congo red; and (6) serological properties. The data now available appear to indicate that strains of unquestioned purity of the typical crowngall organism possess many properties which are different from those of the hairyroot organism. These, when correlated with the distinct kinds of response of the host plants to the invasion of the pathogens, strongly suggest differentiation of a specific rank.

Studies on the differentiation of the crowngall type of bacteria from non-pathogenic bacteria of the radiobacter group. WILLIAM H. WRIGHT, A. J. RIKER, and H. E. SAGEN.

The striking similarity of pure cultures of the typical crowngall organism (*Bact. tumefaciens* Smith and Town.) and *B. radiobacter* Beij. is readily apparent when they are compared. Because both microorganisms may be abundant in the soil and present in or on material used for the isolation of cultures of the crowngall organism, their cultural differentiation without resorting to plant inoculation is desirable.

The two microorganisms have been found to differ in respect to these characters: (1) motility, (2) morphology, (3) absorption of dyes, (4) growth on glycerophosphate media, (5) reduction of nitrates, and (6) serological properties. The crowngall and radiobacter types are very similar in many of the characteristics where the crowngall and hairyroot organisms are different. An effort is being made to discover a satisfactory cultural means of differentiation.

Potency and specificity of a lytic principle (bacteriophage) obtained from Pseudomonas tumefaciens. J. H. MUNCIE and M. K. PATEL.

A lytic principle has been obtained from a pure culture of *Ps. tumefaciens* (V) and also from an artificially induced gall on sugar beet (III). The potency of the lytic principle in each case has been increased until, after 17 filtrations, "phage" V at a dilution of 10^{-14} and "phage" III at 10^{-10} dilution cause lysis of a 24-hour-old broth culture of *Ps. tumefaciens*. Cultures of *Ps. tumefaciens* failed to produce infection on tomato plants, after being subjected to the action of the bacteriophage for nine hours. The lytic principle survives 10 minutes' heating at 80° C. but is inactivated at 85° C.

When tested against 20 strains of *Ps. tumefaciens* from various hosts and eight other bacterial plant pathogenes, "phage" V was found to be specific in its lytic action, affecting only the strain of the crown-gall organism from which it was developed.

Some researches on Pseudomonas tumefaciens. M. K. PATEL.

Immune serum produced against *Pseudomonas tumefaciens* (raspberry strain) by injections of a rabbit agglutinated the homologue up to and including a titre of 1–12800, but not the crown-gall organisms obtained from peach (*Prunus persica*), apple (*Pyrus malus*), geranium (*Pelargonium hortorum*), walnut (*Juglans nigra*), rose (*Rosa odorata*), sand cherry (*Prunus besseyi*), and incense cedar (*Libocedrus decurrens*). The raspberry strain inoculated into weeping willow (*Salix babylonica*) and isolated after more than three months was not distinct from the original strain in agglutination tests. The agglutinable and inagglutinable pathogenic strains of *Ps. tumefaciens* were not affected by

a passage through tomato plants. The crown-gall organism seems to survive passage through the blood stream of rabbits and the intestinal tract of mice and guinea-pigs.

Further agglutination tests with phytopathogenic bacteria. GEORGE K. K. LINK, A. E. EDGECOMBE, and J. G. GODKIN.

The experimental procedure was essentially that reported by Link and Sharp (Bot. Gaz. 83, 1927) and Link and Link (Bot. Gaz. 85, 1928). In addition, absorption of agglutinins was carried out with antisera and suspensions of some of the organisms.

Tests for normal agglutinins were negative. Titres up to 1–10,240 were obtained. The following serological relationships are indicated: *Bact. campestre* is very closely related to *Bact.* X (horseradish pathogen, Lucia McCulloch), less closely to *Bact. cucurbitae, Bact. phaseoli,* and *Bact. translucens; Bact.* X, very closely to *Bact. campestre,* and only distantly, if at all, to the others; *Bact. cucurbitae,* distantly only to *Bact. translucens* var. *undulosum; Bact phaseoli,* closer to *Bact. curcubitae, Bact. translucens,* and *Bact. translucens* var. *undulosum* than to *Bact. campestre; Bact. pruni,* very slightly to only *Bact. cucurbitae* and *Bact. translucens* var. *undulosum; Bact. translucens,* only and only distantly, if at all, to the others; *Bact. cucurbitae,* distantly only to *Bact. translucens* var. *undulosum,* closely to *Bact. translucens* and *Bact.* Z, and very slightly to *Bact. phaseoli; Bact.* Z, very closely to *Bact. translucens* var. *undulosum* and not at all to the others. Data were inconclusive for *Bact.* Y (poppy pathogen, M. R. Bryan).

Physiological studies of Bacterium translucens and Bacterium translucens var. undulosum. JAMES GODKIN.

Bacterium translucens attacking barley and *Bacterium translucens* var. *undulosum* attacking wheat, barley, and rye differ somewhat when grown on agar. Colonies of both organisms when grown on an agar medium (pH 6.8) are circular, smooth, shining, and 1–5 mm. in diameter. The colonies are waxy yellow and have irregular concentric striations within them. Margins are apparently always entire. These descriptions correspond to those given by Jones, Johnson, and Reddy for *Bacterium translucens.* Undulations within the colonies described by Smith for *Bacterium translucens* var. *undulosum* appeared in colonies of both organisms. In *Bacterium translucens* var. *undulosum,* these undulations appeared in 9 days, while in *Bacterium translucens* they appeared in 12 days. They are more pronounced in *Bacterium translucens* var. *undulosum.* In both organisms these undulations seem to disappear in old colonies. No differences were noted when the organisms were grown on potato dextrose and beef-bouillon. No reduction of starch occurred for either organism. *B. subtilis,* used as a check, showed starch reduction. Both organisms produced acid in beef-bouillon plus dextrose, galactose, levulose, and sucrose. In beef-bouillon plus lactose, *Bacterium translucens undulosum* produced an acid reaction, while *Bacterium translucens* produced an alkaline reaction. In beef-bouillon plus maltose both organisms at first produced an alkaline reaction, but later an acid reaction which was more pronounced with variety *undulosum.*

The basal rot of narcissus bulbs caused by Fusarium sp. FREEMAN WEISS.

Though long known to growers, this disease has not received a scientific description. It has caused great losses in planting stock and to bulbs in transit and storage. Affected bulbs suffer immediate decay or may be planted only to rot in the ground or produce feeble, barren shoots. Basal rot was particularly destructive in Virginia and Long Island, N. Y., in 1928.

The prevailing horticultural opinion ascribes this disease to unfavorable conditions during harvesting and storage, admitting to the fungi found in affected bulbs only a

weakly parasitic rôle. Field studies have shown, however, that infection of the roots and basal plate may begin in the field. Subsequent handling and storage conditions only modify the course that this infection takes. Under accepted operating methods losses up to 50 per cent in certain varieties are sustained even by experienced growers.

Typical specimens from all the principal narcissus centers of the United States and from Holland consistently yield a *Fusarium* which belongs to the section Elegans but which is not identified with any described species. Its pathogenicity for narcissus, but for no other flowering bulb or onions, has been repeatedly proved. Injuries favor but are not indispensable to infection. Varieties differ in susceptibility to basal rot.

The relation of the hot-water treatment of narcissus bulbs to basal rot. FREEMAN WEISS.

The hot-water treatment of narcissus against eelworms and bulb flies consists of immersing the bulbs for 2½ hours in water at 110° F. Treatment is required of all narcissus imported into the United States, but cyanide fumigation may be substituted in the case of domestic stocks where fly infestation only occurs.

In this process bulbs affected by Fusarium rot and healthy bulbs are frequently mingled; hence spores and hyphal fragments from the superficial mycelium which develops on affected bulbs may be liberally introduced into the water.

Tests have shown that microconidia of this *Fusarium* retain their viability in water at 110° F. for at least 8 hours. In sweet-potato decoction survival is curtailed and does not exceed 4 hours. Mycelium retains the ability to grow after similar exposure for at least 24 hours. Thus the treatment and subsequent handling may multiply basal-rot infection, a condition frequently realized in practice.

Either the subsequent handling must be such as to suppress infection, such as immediate planting in cool soil, or a fungicide should be introduced into the water to prevent contamination of healthy bulbs. Tests with a number of hot fungicidal treatments show this method to be practicable without injury to the bulbs.

Wire-screen fences for the control of aster yellows. L. O. KUNKEL.

During the past season an attempt was made to control aster yellows by means of screen fences. Adults of the leafhopper *Cicadula sexnotata*, which spreads yellows, are unable to pass through wire screen having 18 meshes to the inch. Such screen was used to fence each of four garden plots 25 feet long and 10 feet wide. The fences, which were 4, 5, 6, and 8 feet high, respectively, were built in the spring before the leafhopper appeared. During the first week of June 45 healthy aster plants were transplanted to each plot. Plants that died were replaced by healthy ones. Yellowed plants were removed from the fenced area as soon as observed. Unfenced aster plantings served as checks.

About one-third of the check plants were yellowed when the disease first appeared in the fenced areas on August 13. Between this date and September 16, when the experiment was ended, only 20 per cent of all fenced plants had contracted yellows, whereas 80 per cent of the check plants were diseased. Under the conditions of the experiment here described, careful rogueing of fenced plots gave fairly satisfactory control of aster yellows.

Wilt-resistant asters. L. O. KUNKEL.

Five strains of the China aster, bearing flowers in a variety of colors and resistant to aster wilt, were grown on plots of aster-sick soil at Yonkers, N. Y. The plots were inoculated four years ago by working compost made from wilted aster plants into the soil. Asters have been grown on the plots during the past five years. The severity of

wilt has continually increased. There was 100 per cent loss from wilt in several commercial varieties during 1928. Four strains grown from seed selected by Mr. D. G. Milbrath and one strain of a commercial variety proved to be very resistant on these plots. There was some loss from wilt in each of the five strains, but they all yielded a good crop of flowers. Seed has been selected from the most resistant plants in each strain. It is hoped that they may be still further improved.

Progress with the control of aster wilt and yellows. L. R. JONES and REGINA S. RIKER.

Selections for resistance to aster wilt, reported upon last year, have been continued with encouraging results. The selections from the favorite red variety, Heart of France, annually tested and reselected since 1925, are apparently stabilized with a high degree of resistance. In the Late Branching type, resistance has also been more readily secured with red than other colors, suggesting association of resistance with this pigmentation. Almost equally promising strains include lavenders and rose, and good beginnings have been made with this and other types in white, pinks, purples, etc.

Previous trials employed small cheesecloth-covered cages to prevent yellows. This year, in trials on a larger scale, a method comparable to shade culture of tobacco was successfully adopted. Hopper-proof cloth-covered inclosures (30 x 100 feet) proved practicable under Wisconsin climatic conditions. Of the tobacco cloths only the finest (22 threads an inch) was effective. Probably slightly coarser, stronger cloths can be specially adapted for hopper exclusion. Since these shaded asters developed superior cut flowers under commercial trials this year, certain commercial florists purpose trying such cloth-shade culture next year.

Iris rust, Puccinia iridis. E. B. MAINS.

Rust from *Iris fulva* has been studied on 14 species of *Iris* which have been listed as hosts. Of the Apogon section of the genus *Iris, I. fulva, I. spuria, I. foliosa,* and *I. missouriensis* were susceptible; *I. ochroleuca* was moderately resistant; and *I. graminea, I. tenax, I. versicolor,* and *I. pseudocorus* were practically immune. The distantly related *I. xiphium* and *I. xiphioides* of the Xiphium section were very susceptible, while *I. dichotoma* of the Pardanthopsis section was moderately susceptible, and *I. tectorum* of the Evansia section and several varieties belonging to pallida and Germanica types of the Pogoniris section were practically immune.

A collection of the rust from the variety Dorothea K. Williamson and another from *Iris versicolor* were found to be physiologically distinct, since *I. fulva* was highly resistant to both of these. *Iris versicolor* is not uniform in its susceptibility to the rust collected on it. A number of plants collected in the immediate vicinity of the susceptible plants proved to be resistant or practically immune.

Anthracnose of the snowberry (Symphoricarpos albus var. laevigatus). W. H. DAVIS.

Diseased fruits were observed on snowberry plants on the campus of the Massachusetts Agricultural College in September. In October, 60 per cent of the fruits had become infected. Examination of diseased fruits showed the presence of acervuli and numerous conidia of a *Gleosporium* (?) sp.

The fungus was isolated, cultured, and Koch's rules of proof fulfilled. When conidia were spread on both punctured and intact surfaces of the fruit, infected areas developed which showed as a wash of cinnamon-rufous color which later turned to shades of hazel, and 5 to 9 days afterwards were dotted with small flesh-colored flecks, acervuli bearing conidia in a manner characteristic of the disease.

In cultures on potato-dextrose agar the floccose mycelium varied from a light mineral to a smoky gray, grew rapidly, and produced dark flecks, the acervuli, with conidia 1 to 2 mm. above the agar surface.

The fungus is described as follows: *Acervuli:* mostly subcutaneous, erumpent, solitary to confluent; width, 100 μ: length, 60 μ (20 measured). *Conidiophores:* Unequal hyaline, unbranched, perpendicular, subclavate, fasciculate, 2 × 20 μ (20 measured). *Conidia:* terminal, cylindrical, apex rounded, hylum slightly protruding, nonseptate, hyaline; limits, 4.5–7.2 × 12.6–20 μ; standard, 5.4 × 16 μ (50 measured); contents, granular, with an opaque area (nucleus) near the center.

Further studies of privet anthracnose. A. J. MIX.

A privet whose botanical relationships are unknown is being offered by nurserymen under the name "Lodense." It was reported to the writer by H. W. Anderson as susceptible to anthracnose. Results of inoculations using *Glomerella cingulata* from privet and apple justify the following conclusions: 1. "Lodense" privet is as susceptible to anthracnose as is European privet (*Ligustrum vulgare*) and must be considered unsuitable for hedge planting wherever the disease occurs. 2. *Glomerella cingulata* from apple is pathogenic to both these privets, but much less so than is *G. cingulata* from privet.

A considerable series of inoculations of apple branches were made with the fungus from both apple and privet. None of these resulted in infection.

Parasitism of Conopholis americana Wallr. on roots of Quercus bicolor Willd. K. D. DOAK.

The growth of *Conopholis americana,* a non-chlorophyllous phanerogamic parasite on roots of *Quercus bicolor,* resulted in a gall-like structure composed largely of parasitic tissue. Sections taken through the gall demonstrated that the glandular, easily separated groups of storage cells of the parasite were enclosed by connective tissue in which lignified conducting vessels were found. At the point of contact between parasite and host tissue, frenate extensions of normal xylem vessels of the latter were found extending into the gall to a distance of 10–12 mm. In each gall examined there were more than 15 such extensions, less than one-half of which coiled backward to form slightly clubbed tips. Abnormal arrangement of host cells occurred in these clubbed projections. Intracellular absorbing organs of the parasite were not present in host cells at the region of direct contact. Cell-sap concentration was found to be least in the host tissue, with sucrose as a plasmolyzing agent. Bacterial or fungal symbionts were not present in these galls.

Biologic control of the aphids Rhopalosihum persicae Sulzer and Aphis gossypii Glover. J. A. B. NOLLA.

Laboratory and field experiments have shown that the aphids which feed on eggplants, peppers, and cucumbers can be effectively controlled by means of the fungus *Acrostalagmus aphidium* (determined by Vera K. Charles, Bureau of Plant Industry, Washington, D. C.). A spore suspension was sprayed on a field of eggplants heavily infested with aphids. This resulted in death of the majority of aphids. Experiments under controlled conditions were made with healthy colonies of the aphids feeding on eggplant, cucumber, and pepper plants, which were sprayed with a spore suspension of the fungus. The method is very cheap and can be applied with great advantage under favorable conditions of humidity.

Further results of oat-smut control in Ohio. A. L. PIERSTORFF and J. D. SAYRE.

In 1927 formaldehyde dust was introduced for the control of smuts of oats. This year, through the Extension Department of the Ohio State University and the Ohio

Agricultural Experiment Station, more data have been obtained on its use under different conditions. Farm demonstrations were conducted where its use under actual farm conditions were shown. The results of these tests on the control of oat smuts in fields of grain were obtained. The Experiment Station continued tests on a smaller scale in the greenhouse and in the field. Different methods of treating the grain and various concentrations of the dusts were used at three dates of planting. The results led to the following conclusions: that a 5 per cent formaldehyde dust at the rate of about 3 ounces per bushel of grain is most effective; that the treatment need not be made in a closed container; and that the grain should be left in sacks over night before sowing.

The rôle of humidity in the life cycle, distribution, and control of the loose-smut fungus of wheat. V. F. TAPKE.

The wheat varieties, Little Club, Jenkin, and Washington Hybrid 128, when continuously grown in the Pacific Coast States, rarely contain loose smut, but these wheats proved extremely susceptible when the flowers were artificially inoculated in a humid greenhouse at Arlington Experiment Farm, Rosslyn, Va. The loose-smut fungus of wheat infects only through the flowers of its host. At infection time the relative humidity of the atmosphere in the Pacific Coast States usually is very low. Through the use of chambers with definite relative humidities it has been found that the spores do not germinate within the flowers at the low humidities which normally prevail on the Pacific Coast when the wheats are in bloom, thus preventing infection. The flower-infecting loose smuts of oats and barley as well as the loose smut of wheat are rarely found in those sections of the country where the atmosphere at flowering time is very dry, although the host is widely grown. Loose smut in wheat grown on irrigated land was reduced but not controlled by withholding water at flowering time.

Studies of certain soil phases of the wheat take-all problem. HURLEY FELLOWS.

Experiments have shown that *Ophiobolus graminis* is present to a depth of at least 10 inches in infested soils in Kansas. However, in inoculation experiments, the most severe infections resulted when inoculum was placed only one inch below the crowns of the wheat plants. When the inoculum was placed 4 or more inches below the crowns, the wheat plants were uninjured.

Experiments have shown that inoculum of *Ophiobolus graminis* grown on a mixture of autoclaved oats and barley soon loses its virulence when the entire culture is added to uninfested soil. Each of several kinds of organic matter such as chicken manure, horse manure, alfalfa stems and leaves, boiled barley-oat mixture, and potato flour added separately to infested soil, both in greenhouse and field, greatly reduced severity of take-all. In some cases almost perfect control was obtained. (Cooperative investigations between Kansas Agricultural Experiment Station and Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Some chemical and morphological phenomena attending infection of the wheat plant by Ophiobolus graminis Sacc. HURLEY FELLOWS.

Ophiobolus graminis penetrates directly through the epidermis of the roots and subcoronal internode and coleoptile of the wheat plant. The crown becomes infected through the subcoronal internode. All portions of the tissue attacked may be invaded.

Certain cell walls in the vicinity of the invading parasite become thickened or produce elongated protuberances in front of and around the invading hyphae. These structures, previously undescribed, are given the name "lignitubers." The xylem walls of the subcoronal internode may become so thickened that plugging occurs. In the

coleoptile, the thickenings occur mostly in the epidermis while in the roots they are scattered promiscuously.

Other cells, however, are completely disintegrated. This is especially true of phloem and adjoining cells. With the exception of the xylem, the entire stele of the roots may be disintegrated.

Microchemical studies show a non-uniform reduction of cellulose in diseased roots. The cellulose loss is replaced by lignin and a slight amount of suberin. This is true especially of the thickened walls and the "lignitubers." Other substances are not changed by the invasion of the fungus. (Cooperative investigations between Kansas Agricultural Experiment Station and Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Relative susceptibility of various varieties of sorghum to rust, Puccinia purpurea. E. B. MAINS.

Greenhouse and field tests of several varieties of sorghum at Lafayette, Indiana, during 1928 showed marked differences in the reaction of varieties. All varieties of the Sorgo group included in the experiment were more or less susceptible, differing principally in the number of uredinia developed. Dwarf Sumac, Fielding Sumac, and Red Amber proved very susceptible; and Leoti Red, Freed, White African, and Kansas Orange were moderately susceptible. Of the grain sorghums, all of the five strains of Feterita tested were very susceptible. Of the Kafirs, Red Kafir and Sunrise were very susceptible, Dawn was moderately susceptible, and Blackhull and Reed were moderately resistant. All five of the strains of Milo were very resistant, showing only small purplish spots with an occasional small uredinium. Two strains of broomcorn and two of Hegari were moderately susceptible. A line of Dwarf Shantung Kaoliang and one of Shallu showed considerable resistance, especially in the field. Infection of almost all of the lines, resistant as well as susceptible, produced a purpling of the infected areas. One variety, the susceptible Leoti Red Sorgo, was outstanding on account of the absence of purple in infected spots. (Cooperative investigations between the Purdue Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau Plant Industry, U. S. Department of Agriculture.)

Preliminary results from cross inoculation and culture studies upon the fungus Rhynchosporium secalis (Oud.) Davis causing scald of cereals and other grasses. RALPH M. CALDWELL.

The organism *Rhynchosporium secalis* (Oud.) Davis which causes scald of barley, rye, and other grasses, has been isolated from five different hosts growing in the vicinity of Madison, Wisconsin, and successfully grown in culture. Besides the cereals, barley and rye, isolations have been made from the grasses, *Agropyron repens, Bromus inermis,* and *Hordeum jubatum.* The last named plant has not previously been reported as attacked by this fungus. Single spore cultures of the fungus from barley are distinct from those from rye in pigmentation, type of growth, and relative abundance of conidia, these characters being very constant.

Oderbrucker barley, Schlansted rye, and Marquis wheat have been inoculated with these single-spore cultures by atomizing conidial suspensions over the leaves of seedling plants. Infection was secured only in cases where the plant inoculated was the host from which the culture had originally been secured. Very typical lesions were obtained where this was the case. Similarly, only barley became infected where overwintered diseased barley leaves were used as a source of inoculum.

It seems quite evident that barley and rye in the vicinity of Madison, Wisconsin, are each attacked by a distinct physiologic form of this fungus.

Further studies on Penicillium injury to corn. HELEN JOHANN.

Various pedigreed strains of dent corn in the field at Bloomington, Illinois, and Madison, Wisconsin, in the summer of 1928, showed marked reduction in stand and yield following inoculation at time of planting with a spore suspension of *Penicillium oxalicum*. Symptoms in the field were similar to those previously reported in the greenhouse.

The parasite is not aggressive enough to enter vigorous, living cells. Sections through lesions indicate that a toxic substance injures or kills the cells in advance of the fungus. Oxalic acid produces similar lesions when applied to the surface of the seedling. Yellowing and drying of leaves, symptoms of the Penicillium disease, were also reproduced by placing young seedlings in dilute solutions of oxalic acid.

Chemical analysis disclosed the presence of considerable quantities of oxalic acid in corn-seedling decoctions on which *P. oxalicum* had grown for six days at 20° C.

The fungus is chiefly intercellular, and not confined to any particular tissue. In the most severely injured seedlings it was found in the region of the cotyledonary plate. The hyphae entered by working their way between the cells of the root tip, through the depleted scutellum, the ruptured coleorhiza, root ruptures, or injured tissue of any sort. (Cooperative investigations between the Office of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, the Wisconsin Agricultural Experiment Station and Funk Brothers Seed Company.)

Reaction in agronomic strains of timothy to Ustilago striaeformis (Westd.) Niessel. W. H. DAVIS.

An investigation was undertaken to answer the following: 1. Are some agronomic strains of timothy (a) susceptible to *Ustilago striaeformis*, (b) resistant, or (c) immune? 2. Are there physiologic forms of the fungus parasitizing this host?

Timothy seeds were collected from the following number of stations: in Wisconsin, 4; New York, 3; Iowa, 2; Connecticut, 1; Massachusetts, 8; New Hampshire, 1. Agronomic strains 4081, 4001, 4123, 4003, 4031, 4078, 4079, 4059, 1777, 1676 were supplied by Dr. C. H. Meyers, of Cornell.

The spore materials were collected from: (1) 5 field stations in Massachusetts, 2 in Connecticut, and 1 in New York; (2) infected timothy plants which had been inoculated with spores collected at stations in Wisconsin and New York.

Infected plants were observed among partly matured, inoculated plants, the seedlings of which had been cultured from seeds collected at each of the field stations and in each agronomic strain. The agronomic strains were arranged in order of their resistance beginning with the least resistant: 4079–R1, 1676–R2, 4123–R3, 4001–R4, 1777–R5, 4081–R6, 4003–R7, 4078–R8, 4031–R9, 4059–R10. Artificial infections varied from 70 to 1 per cent. No agronomic strains of timothy were found immune to the spore form of *U. striaeformis* used, and no physiologic strains of the fungus were detected.

Studies of cold resistance and susceptibility in corn. J. R. HOLBERT and W. L. BURLISON.

Wide variations in resistance to injury from cold in the fall have been found to exist in inbred strains of corn, in recombinations of inbreds, and in commercial open-pollinated strains, as they approach maturity. Some inbreds were killed by a few hours exposure to temperatures ranging from 45° to 50° F. Many inbreds, recombinations, and commercial strains were injured or killed by a succession of cold nights during which temperatures ranged from 45° to 33° F. Other inbreds and recombinations withstood six hours of 32° to 30°, and a few others withstood 28° F. for hours. These differences have been observed under natural conditions for the past five years and some of them were found under accurately controlled artificial conditions in the field in 1928.

In the spring of 1925, when field temperatures dropped below 32° for several hours, reaching as low as 25° F., a few inbreds and recombinations, 4 to 6 inches high, escaped without apparent injury. Under the same conditions many other strains were severely injured and some never recovered from the injury.

Seed, matured on plants high in cold resistance, produced plants more resistant to seedling blights, lodging, and stalk breaking than did seed gathered at the same time from corresponding plants previously injured by cold. (Cooperative investigations between Illinois Agricultural Experiment Station, Funk Brothers Seed Company and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.)

Mutation in Ustilago zeae. E. C. STAKMAN, J. J. CHRISTENSEN, and W. F. HANNA.

Ustilago zeae is very unstable genetically. Mutation occurs with surprising frequency in some monosporidial lines, which apparently are haploid clones; while it occurs but rarely in others. During a period of 9 months, about 100 different mutants were produced by one line and more than 70 by another. That these mutants are not mere temporary phenotypes is shown by their constancy; that they are not the result of normal segregation is indicated by cytologic and genetic studies. The mutants differ from their parents and each other in one or more of the following respects: morphology, cultural characters, and apparently sometimes in sex—therefore, in potential pathogenicity. The frequency of mutation can be influenced by certain environmental conditions, and suitable media sometimes are necessary to bring out the differences between the mutants.

Calcium monosulphide, a substitute for lime-sulphur for summer spraying. R. H. HURT.

Experiments conducted on Winesap apples for the past three years have shown that calcium monosulphide is as efficient as lime-sulphur in the control of fungous diseases of apples and is much more safely and easily used. Calcium monosulphide has caused no injury, whereas lime-sulphur and bordeaux mixture caused severe injury to foliage in adjoining plots. It also gave a better finish to the fruit.

In the 1926 and 1927 experiments calcium sulphide U. S. P. containing approximately 60 per cent calcium monosulphide was used at the rate of 12½ pounds to 50 gallons of water. In the 1928 trials, a commercial preparation containing approximately 65 per cent calcium monosulphide was used at the rate of 10 pounds to 50 gallons of water.

When calcium monosulphide was used as a complete summer spray the average scab infection for the three-year period was 3.19 per cent. When lime-sulphur was used for the early summer applications followed by bordeaux mixture, the average scab infection for the three-year period was 7.40 per cent. The three-year average of scab infection in the checks was 75.33 per cent.

A promising spray for the control of peach bacterial spot. JOHN W. ROBERTS and LESLIE PIERCE.

In southern Indiana a spray composed of zinc sulphate 4 lbs., hydrated lime 3 lbs., casein lime ½ lb. or zinc sulphate 4 lbs., hydrated lime 4 lbs., alum (aluminum ammonium sulphate) 1½ lbs. to water 50 gals., has checked infection of the peach by *Bacterium pruni.* In 1928 six applications were made at intervals of two weeks beginning at petal-fall. Care was taken to cover fruits and the underside of leaves. Spotting of leaves and defoliation were markedly less on these trees, and the leaves were a deeper green and larger than those of trees sprayed or dusted with sulphur.

The number of infected fruits was not greatly reduced but the number and size of infections were much less. From the zinc plats 9 and 17 per cent respectively of

the fruit were culled because of *Bacterium pruni* infections, and 27 per cent from the unsprayed plat. Less than 1 per cent of the unsprayed fruits was wholly free of infection while in the zinc plats 10 and 5 per cent were free respectively.

Used alone, the zinc sprays caused no noticeable injury, but the addition of arsenate of lead caused injury enough to suggest caution.

Phony disease of the peach. LEE M. HUTCHINS.

Phony disease of the peach is a communicable disease, the cause of which is not definitely determined. The infective principle apparently resides only in the roots, and the incubation period is about 18 months. Since its first recorded observation at Marshallville, Ga., about 50 years ago, phony disease has spread until at the present time it occurs in the districts that produce 90 per cent of the commercial peach crop of Georgia. It has recently appeared also in eastern Alabama. Phony trees produce short terminal growth, profuse lateral branching, and deep green foliage. Trees never recover from the phony disease, neither do they die of it, but live on for many years. Economic losses are due to a marked dwarfing effect and to a decrease in the average size of the fruits and in the number of fruits per tree. After a few years of the disease phony trees bear few if any marketable peaches. Extensive investigations with a view to developing control measures based on the selection of resistant stocks are under way at the United States Peach Disease Field Laboratory, Fort Valley, Ga.

The relation of certain bacteria to the development of roots. A. J. RIKER, W. M. BANFIELD, and G. W. KEITT.

Root stimulation and the development of some types of "hairyroot" have been shown to follow inoculations with certain bacteria. These results have been obtained through continuation of studies (1) of malformations on apple nursery stock which were not prevented by the control measures used and (2) of certain organisms which closely resembled *Bact. tumefaciens* Smith and Town. in colony characters on agar media and which were found associated with certain overgrowths on apple. Hairyroot has followed inoculations through punctures in the underground parts of the current season's growth from apple grafts with approximately 40 strains (isolations from 40 sources) of bacteria isolated from overgrowths on apple. In the early stages overgrowths developed which were more or less conspicuous and from which fleshy roots appeared. In the later stages the fleshy roots may become fibrous. In some cases the enlargements at the bases of the root developments were quite conspicuous. Parallel inoculations with known *Bact. tumefaciens* resulted in typical crowngalls and in no case showed any stimulation of roots. From hairyroot induced by different inoculations, organisms have been repeatedly isolated which apparently are identical with those inserted. Inoculations with six reisolated strains have in turn produced hairyroot. Root stimulation has also followed inoculations on rose and honeysuckle.

Progress in freeing the virus of mosaic disease of tobacco from accompanying solids. C. G. VINSON and A. W. PETRE.

The virus of mosaic disease of tobacco may be precipitated from solution by means of an aqueous solution of safranin. The virus may be recovered by removing the safranin from the precipitate.

Material which gives an infectious solution when redissolved in water has been salted out of infectious juice with ammonium sulfate and also with magnesium sulfate.

Two volumes of acetone or alcohol, when added to one volume of juice from diseased plants, at about 0° C., throws down a precipitate containing practically all the virus.

Some of the phosphate, sulfate, and most of the protein and pigment may be removed from juice of diseased plants, with low concentrations of lead acetate and barium acetate

Cleared juice from diseased plants, concentrated in vacuo to 0.4 the original volume and brought to about 0° C. gives a precipitate when two volumes of acetate at −15° C. are added. This precipitate contains only about 10 per cent of the solids of the original juice, but apparently contains all of the original virus.

The virus behaved in many ways similar to a chemical substance.

The separation from mosaic tomato plants of toxins which produce some of the typical mosaic symptoms. H. R. KRAYBILL, P. H. BREWER, R. W. SAMSON, and M. W. GARDNER.

Tomato plants inoculated with a combination of the non-infectious filtrate (Kraybill and Eckerson, Amer. Jour. Bot. 14: 487–495. 1927) from diseased plants, which produced fern-leaf symptoms, and juice from potato tubers in no case produced the streak disease. Plants inoculated with the residue, which contains the tomato mosaic principle, and juice from potato tubers developed tomato streak disease. Numerous inoculations of tomato plants with the filtrate freed from the infectious mosaic principle have produced the fern-leaf-like symptoms. Repeated efforts to transfer by inoculation from these fern-leaf plants to healthy tomato plants have been negative. These filtrates have been heated for two and a half hours at 126° C. without any reduction in their activity. Frequently these tomato plants which show the fern-leaf-like symptoms produced by the non-infectious filtrates eventually outgrow these effects. Inoculations with filtrates prepared from healthy tomato plants have failed to produce these fern-leaf-like symptoms. The results show that the material in the filtrates which produce these fern-leaf-like symptoms is non-living, is of the nature of heat stable toxins, and is probably produced by the mosaic virus.

Purification and certain properties of the tomato mosaic virus. P. H. BREWER, H. R. KRAYBILL, and M. W. GARDNER.

Juice from mosaic tomato plants thoroughly ground in a food chopper is expressed through heavy muslin in a Cossette porcelain press, diluted with an equal volume of distilled water, and passed through a supercentrifuge three times. The liquid is discarded. The dark green gummy residue is scraped from the bowl of the centrifuge, resuspended in distilled water by stirring with a Bouyoucos deflocculator, and again passed through the supercentrifuge. To the liquid, which is somewhat yellowish and cloudy, aluminum gel prepared so as to have a slightly acid reaction is added. The mixture is filtered and the filtrate is a clear and colorless suspension of the virus apparently as active as the originally expressed juice of the plant. The aluminum gel removes the yellow color and the suspended solids. The longevity of the virus in this state at ordinary temperatures is less than two months. The virus is inactivated when, by the addition of NaOH, the suspension is made more alkaline than pH 7.7, and becomes active again at about the same point when HCl is added.

Progress report on cereal scab development during the season of 1928. JAMES G. DICKSON, E. B. MAINS and HELEN JOHANN.

Scab has again taken a great toll of small grains in the central states. The losses were greater in spring wheat, barley and oats than in 1919. In northern states large amount of damage was done by spread of the disease in shocks.

Clean, well plowed fields are good assurance against reoccurrence of an epidemic next year. Of wheat varieties tested, Norka, Progress, Resaca, and Illinois 1 have shown the greatest resistance to scab. (Cooperative investigations between Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Wisconsin and Purdue Agricultural Experiment Stations.)

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THE PEACH APHID (MYZUS PERSICAE SULZ.) AS AN AGENT IN VIRUS TRANSMISSION[1]

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INTRODUCTION

Following the report by Allard (1) of the transmission of tobacco mosaic by aphids, increasing attention has been paid to the relation of insects to plant virus diseases. Numerous investigations in recent years have demonstrated the ability of various species of aphids and leafhoppers especially to transmit certain viruses. Apart from the peculiar scientific interest involved in these relationships, considerable importance has been attached to such insects as agents in virus dissemination, and the rôle played by them in disease epidemics has been strongly emphasized. The peach aphid (*Myzus persicae* Sulz.), in particular, has been reported as capable of transmitting several apparently distinct diseases of this group (5, 11, 15, 16, 17, 18, 19).

As a result of several years' work at the Wisconsin Agricultural Experiment Station on the overwintering and spread of tobacco mosaic in the field, the importance of aphids in the dissemination of this particular disease has, however, been questioned (13). A detailed investigation of this phase of the problem has consequently been undertaken. While the work naturally involves a consideration of more than one species of aphid in relation to virus transmission, the present paper is limited to a report of certain greenhouse studies of a single species only, namely, the green peach aphid, *Myzus persicae* Sulz. This aphid occurs commonly upon tobacco and other plants in the greenhouse, and is one of two species previously reported by Allard (3) as active carriers of tobacco mosaic.

[1] Cooperative investigations of the Wisconsin Agricultural Experiment Station and the Office of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

[2] I wish to acknowledge gratefully my indebtedness to Dr. James Johnson, at whose suggestion this work was undertaken, for his advice and encouragement throughout the course of the investigation; to Dr. S. P. Doolittle and Dr. C. F. Lackey for furnishing specimens of *Myzus persicae;* and to Dr. A. A. Granovsky for verifying the identification of the species.

MATERIAL AND METHODS

Since a large number of solanaceous plant species in addition to tobacco are known to be susceptible to tobacco mosaic (1), and since some of these have been reported as wild hosts of the virus (1, 9), it seemed desirable to include several other species beside tobacco in a study of the transmission of this mosaic. For the sake of comparison, it was decided to investigate also the transmission of a second mosaic disease between the same host species in parallel trials. Cucumber mosaic has been shown to be transferable to a number of solanaceous host plants by the method of artificial juice inoculation (8, 12); this disease was selected for comparative study.

In the actual choice of plant species, it was therefore necessary to consider two factors: (1) susceptibility of the species to both viruses, and (2) suitability as a food plant for the peach aphid. The following six species were finally selected: tobacco (*Nicotiana tabacum*), *N. rustica, N. glauca, N. glutinosa,* pepper (*Capsicum annuum*), and ground cherry (*Physalis pubescens*). These plants are all more or less easily infected with either virus by methods of artificial inoculation, and the peach aphid feeds and multiplies readily upon them all. Furthermore, with two exceptions, both viruses are readily recoverable from infected plants by artificial means. In the case of *N. glutinosa,* however, it has been shown by Allard (1) and by Johnson (12) that, although tobacco mosaic may have a marked effect upon this host, the plant juices are apparently not infectious. On pepper, too, although cucumber mosaic produces typical mosaic symptoms, yet the virus is rarely, and with difficulty, recoverable from this host by artificial means; in other words, pepper is a poor "source of inoculum" (14) for the cucumber mosaic virus. These two hosts were included, however, with a view to determining whether or not they would behave as equally poor sources of inoculum in respect to aphid transmission.

The two viruses were supplied by Dr. James Johnson, that of tobacco mosaic [*Tobacco virus 1* (14)] coming originally from a diseased tobacco plant on the University grounds at Madison and that of cucumber mosaic [*Cucumber virus 1* (14)] from a mosaic cucumber plant furnished by Dr. S. P. Doolittle, United States Department of Agriculture. Both were maintained by repeated transfer on tobacco in the greenhouse.

Specimens of the peach aphid were taken from some tobacco plants in the greenhouse and the identification verified by Dr. A. A. Granovsky of the Wisconsin Experiment Station. These aphids proved to be non-viruliferous. Subsequent colonies reared upon cabbage in an insect-proof cage furnished a source of non-viruliferous aphids which was employed throughout the investigation. The aphids appeared to thrive particularly well upon this host and multiplied with great rapidity on it. The colonies con-

sisted of the "summer generations" or yellowish-green, viviparous females, the majority apterous and a low, somewhat variable, proportion winged. So far as could be determined, no other forms of this aphid were present at any time. Since cabbage is not susceptible to either tobacco or cucumber mosaic, there was little danger of these colonies becoming infected with either virus. Nevertheless, their condition in this respect was determined from time to time by transferring a number of individuals to healthy, young tobacco plants. All such tobacco plants remained healthy, indicating that the aphids did not carry any transmissible virus.

In studying the transmission of the two viruses, the different hosts were infected with each mosaic disease and large numbers of aphids colonized upon them in separate cages. After five to eight or more days, the aphids were transferred to healthy plants of the species required for investigation. In all transmission experiments, only young, vigorously growing plants were used, and these were handled in sets of five. The aphids were transferred from the mosaic host by removing one or more of the aphid-bearing leaves and laying them on the healthy plant. A piece of paper was usually placed under such leaves to prevent any contact with the healthy plant. About 30–40 or more adult aphids, together with a large number of nymphs, were transferred in this manner to each plant; these soon migrated to the new host, and the mosaic leaf and paper were subsequently removed with sterile forceps. The aphids fed and multiplied abundantly upon the new hosts in all cases. Usually parallel transfers were made simultaneously for each virus. In each trial, an equal number of plants was treated in a similar manner as a control, the mosaic leaves in this case being, however, entirely free from aphids. The plants were placed after treatment in a large glass chamber divided into insect-proof sections, the infested plants and the controls being kept in different sections. The temperature of the chamber normally ran from 75° to 80° F., but occasionally varied from about 70° to 90° F. After about a week, the plants were brought out into a warm greenhouse (80°–90° F.), which was immediately fumigated. This greenhouse was fumigated regularly twice a week to keep it free from aphids, and no infested plants were brought into it except immediately before fumigation. The plants were discarded after two to three weeks, since any mosaic symptoms appearing after this time would not be accepted as resulting from primary aphid infection.

EXPERIMENTAL RESULTS

It is not believed necessary to enter into a full description of the symptoms produced by the two viruses on the different host plants employed. The chief differential symptoms have been described by Johnson (14), and

the reader is referred to this author for further details. It should be emphasized, however, that the two diseases are readily distinguishable upon all six hosts when young plants are used. Generally speaking, cucumber mosaic on these host plants is characterized by marked chlorosis, malformation, and mottling; tobacco mosaic by marked stunting and (tobacco and *N. glauca* excepted) by a progressive necrosis of the plants. In any case of doubt as to diagnosis, juice inoculations were made to tobacco, when the virus could be readily identified.

The results of the comparative transmission experiments with *Myzus persicae* are summarized in table 1. It will be seen from this table that transmission of the cucumber mosaic virus by the aphid was readily secured among all six hosts, 100 per cent infection commonly occurring in different trials. Of a total of 370 plants tested, 345 became infected with cucumber mosaic, as compared with 8 out of 370 plants in the control series (Plate I, B, C, E, F, H, J and Plate II, B, C, E, F, H, J). Aphid transmission of this virus was also obtained to tomato (*Lycopersicon esculentum*) from tobacco, *N. rustica,* and tomato, and to cucumber (*Cucumis sativus*) from tobacco and pepper. In the latter transfers, it was interesting to note that although the aphids attempted to feed upon the young cucumber plants, they did not stay on this host for any length of time, and after 48 hours all had died or disappeared. Nevertheless, 100 per cent infection was obtained in each trial, all control plants remaining healthy.

It will be observed that the percentage of infection resulting in the different trials varied very little with the species of host employed, whether this served as a source of the virus or as a host to which the virus was transmitted. Pepper in particular, though a very poor source of inoculum for cucumber mosaic virus as far as artificial juice inoculation is concerned, proved to be as suitable a source of inoculum with respect to aphid transmission as any of the five other hosts employed. Again, while these hosts have been found to differ considerably in relative susceptibility to infection with cucumber mosaic through artificial means, they appear to be all about equally susceptible to infection by aphids. For example, while it is comparatively easy to obtain 100 per cent infection of tobacco with cucumber mosaic by methods of artificial inoculation, this has rarely or never been obtained by the writer on certain other hosts such as pepper, *N. glauca,* and *N. glutinosa.* Comparable results have been obtained by Johnson (14). On the other hand, equally high percentages of infection resulted from aphid infection on all hosts studied.

Infection by artificial means was most readily secured on these less susceptible hosts by repeatedly pricking viruliferous juice into the midribs

TABLE 1.—*Comparative trials of the transmission of the cucumber and tobacco mosaic viruses by Myzus persicae*[a]

Mosaic host (source of virus)	Mosaic virus	Hosts												Total infections	
		N. tabacum		*N. glauca*		*N. glutinosa*		*N. rustica*		*C. annuum*		*P. pubescens*			
		Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
Nicotiana tabacum	Cucumber	30/26	30/1	10/9	10/0	10/10	10/0	15/15	15/0	15/15	15/0	15/14	15/0	95/89	95/1
	Tobacco	30/0	30/0	10/0	10/0	10/0	10/0	20/1	20/1	15/0	15/0	15/0	15/0	100/1	100/1
N. glauca	Cucumber	15/13	15/0	10/10	10/0	5/3	5/0	10/10	10/0	10/9	10/0	10/10	10/1	60/55	60/1
	Tobacco	15/0	15/0	10/0	10/0	5/0	5/0	10/0	10/0	10/0	10/0	10/0	10/0	60/0	60/0
N. glutinosa	Cucumber	5/4	5/0	5/4	5/0	5/5	5/0	10/9	10/1	5/5	5/0	5/4	5/0	35/31	35/1
	Tobacco	5/0	5/0	5/0	5/0	5/0	5/0	10/0	10/1	5/0	5/0	5/0	5/0	35/0	35/1
N. rustica	Cucumber	15/12	15/0	10/9	10/0	5/5	5/0	10/10	10/2	10/10	10/0	15/15	15/0	65/61	65/2
	Tobacco	15/0	15/0	10/0	10/0	5/0	5/0	10/0	10/0	10/0	10/0	10/0	10/0	60/0	60/0
Capsicum annuum	Cucumber	10/8	10/0	10/10	10/0	5/5	5/0	10/10	10/1	10/8	10/0	10/8	10/0	55/49	55/1
	Tobacco	10/0	10/0	10/0	10/0	5/0	5/0	10/0	10/0	10/0	10/0	10/0	10/0	55/0	55/0
Physalis pubescens	Cucumber	15/15	15/1	10/10	10/0	5/5	5/0	10/10	10/0	10/10	10/0	10/10	10/1	60/60	60/2
	Tobacco	20/2	20/0	10/0	10/0	5/0	5/0	30/6[b]	30/1	10/0	10/0	10/1	10/0	85/9	85/1
Total infections	Cucumber	90/78	90/2	55/52	55/0	35/33	35/0	65/64	65/4	60/57	60/0	65/61	65/2	370/345	370/8
	Tobacco	95/2	95/0	55/0	55/0	35/0	35/0	90/7	90/3	60/0	60/0	60/1	60/0	395/10	395/3

[a] 'Infested' denotes those plants to which aphids were transferred from a mosaic host; 'control' denotes those plants which were treated with osaic leaves free from aphids and which served as controls to the infested plants. In all fractions, the numerator represents the total number of ants either infested with aphids or treated as controls, as the case may be; the denominator the number of such plants which developed symptoms mosaic.

[b] Four of these infections probably resulted from handling of the plants (see text).

of the leaves; the incubation period was then relatively short and about the same as that resulting from aphid infection, the minimum observed in each case being six days. When viruliferous juice was pricked or scratched only into the lamina of the leaves, however, an extremely low percentage of infection resulted, together with a somewhat longer incubation period. Since the aphids appeared to feed usually upon the larger veins of the leaf, this may in part account for the higher percentages of infection produced by them. In any case, it is evident that the peach aphid is a highly efficient agent of transmission of cucumber mosaic among certain solanaceous host plants and, generally speaking, a more reliable means of securing infection than present methods of artificial inoculation. Doolittle (7) reports a similar situation in respect to the transmission of the same mosaic disease by the melon aphid (*Aphis gossypii* Glover).

Contrary to previous expectation, no evidence was obtained from these experiments of the transmission of tobacco mosaic by the peach aphid among any of the host plants employed (Plate I, A, D, G and Plate II, A, D, G). Of a total of 395 plants tested, only 10 developed symptoms of the disease, as compared with 3 out of 395 plants in the controls (table 1). These few cases of infection are believed to arise from certain sources other than aphid transmission, probably from contact with infectious materials of different kinds. In one of the earlier trials of the transmission of tobacco mosaic from *Physalis* to *N. rustica,* the infested plants were handled on one occasion with unsterilized forceps and four of the five plants subsequently became mosaic. Frequent repetitions of this transfer, conducted with the utmost care, resulted in only a single case of mosaic in 20 plants; hence the preceding infections were not regarded as an indication of aphid transmission. Tobacco mosaic is known to be extremely infectious through various means of contact, and *N. rustica* appeared to be even more susceptible than tobacco in this respect. The incident quoted above serves to illustrate this point. It will be noted that 10 of the 13 cases of tobacco mosaic developing on both infested and control plants occurred upon *N. rustica.* Occasional infections were noted also on the plants employed in the cucumber mosaic trials. In any case, the percentage of infection obtained in the trials with tobacco mosaic was exceedingly low (2.6 per cent) and of the same order as that occurring in the controls (0.8 per cent). When compared with the percentage of infection obtained with cucumber mosaic (93 per cent), it is evident that the results should not be interpreted as indicative of any transmission by aphids of the tobacco mosaic virus.

It is not believed that the failure of the aphid to transmit this virus is due to any fault in technique, on account of the ready transmission of the cucumber mosaic virus obtained by the same methods and under identical

conditions. Nor is it believed to be due to the use of insufficient numbers of aphids, since, in various trials, 60 or more adults, together with a large number of nymphs, were transferred to each host plant without any infection resulting, while, on the other hand, preliminary experiments have indicated that as few as five adults alone may be sufficient to cause infection with cucumber mosaic. In regard to the duration of feeding on the mosaic host, in some cases the colonies remained on such hosts for as long as two weeks before transference to healthy plants, and again no infection resulted.

Although Allard (1) has shown that the tobacco mosaic virus is distributed throughout the vegetative parts of infected plants, there remained a possibility that the virus was not present, in sufficient quantity to cause infection, in the actual leaves upon which the aphids had been feeding while on the mosaic hosts. In order to examine this possibility, in a number of trials selected at random, juice inoculations were made to tobacco from each aphid-bearing leaf transferred from the mosaic to the healthy host, as soon as the aphids had migrated from it. The results, which are shown in table 2, clearly demonstrate that in all cases the virus was present in these leaves in sufficient quantity to produce infection through artificial inoculation, although no aphid transmission took place.

Two other strains of *Myzus persicae* have been tested in relation to the transmission of the two viruses and similar results have been obtained with each. One of these strains, taken on sugar beet at Riverside, California, was received from Dr. C. F. Lackey, U. S. Department of Agriculture; the other, taken on tomato at Bradenton, Florida, from Dr. S. P. Doolittle. While only limited trials were conducted in these cases, the results secured are sufficiently decisive to indicate that strains of the peach aphid from different localities may be expected to behave alike in respect to the transmission of the two viruses under consideration. These results are given in table 3.

More conclusive evidence, perhaps, was furnished by the following experiment. It seemed reasonable to assume that if the peach aphid is incapable of transmitting the tobacco mosaic virus, then colonies, reared upon plants infected simultaneously with both tobacco and cucumber mosaic, should transmit only the cucumber mosaic virus from the combination. An experiment designed to test this hypothesis has in fact demonstrated a definite selectivity in virus transmission by this aphid. Aphids were colonized upon young tobacco plants which had been artificially inoculated with both viruses and which showed definite symptoms of the combination disease. After seven days, the aphids were transferred to healthy tobacco plants by the usual leaf method and an equal number of plants were treated as controls with mosaic leaves free from aphids. When

TABLE 2.—*A comparison of the transmission of tobacco mosaic by Myzus persicae with artificial inoculation from the leaves on which the aphids were feeding when transferred from the mosaic host*[a]

Aphid transmission			Artificial inoculation		
Mosaic host (source of virus)	Host to which aphids transferred	Results	Mosaic host (source of virus)	Host inoculated with juice from aphid-bearing leaves[b]	Results
Nicotiana tabacum	*Physalis pubescens*	5/0	*N. tabacum*	*N. tabacum*	5/5
do	*N. rustica*	10/1	do	do	10/10
do	*N. tabacum*	10/0	do	do	10/10
N. glauca	*N. rustica*	5/0	*N. glauca*	do	5/5
Capsicum annuum	*C. annuum*	5/0	*C. annuum*	do	5/5
do	*N. tabacum*	5/0	do	do	5/5

[a] In all fractions, the numerator represents the total number of plants either infested with aphids or artificially inoculated, as the case may be; the denominator the number of such plants which developed symptoms of mosaic.

[b] The "aphid-bearing leaves" are those on which the aphids were feeding when transferred from the mosaic to the healthy hosts.

TABLE 3.—*Comparative trials of the transmission of the cucumber and tobacco mosaic viruses by strains of Myzus persicae from different localities*[a]

Source of aphids	Mosaic host (source of virus)	Mosaic virus	Hosts						Total infections	
			N. tabacum		*C. annuum*		*N. glauca*			
			Infested	Control	Infested	Control	Infested	Control	Infested	Control
Bradenton, Fla.	*Nicotiana tabacum*	Cucumber	10/10	10/0					10/10	10/0
		Tobacco	10/0	10/0					10/0	10/0
	Capsicum annuum	Cucumber	5/5	5/0	5/5	5/0			10/10	10/0
		Tobacco	5/0	5/0	5/0	5/0			10/0	10/0
	Total infections	Cucumber							20/20	20/0
		Tobacco							20/0	20/0
Riverside, Cal.	*N. tabacum*	Cucumber	5/4	5/0			5/4	5/0	10/8	10/0
		Tobacco	5/0	5/0			5/0	5/0	10/0	10/0
	C. annuum	Cucumber	5/5	5/0	5/5	5/0			10/10	10/0
		Tobacco	5/0	5/0	5/0	5/0			10/0	10/0
	Physalis pubescens	Cucumber	5/5	5/0					5/5	5/0
		Tobacco	5/0	5/0					5/0	5/0
	Total infections	Cucumber							25/23	25/0
		Tobacco							25/0	25/0

[a] 'Infested' denotes those plants to which aphids were transferred from a mosaic host; 'control' denotes those plants which were treated with mosaic leaves free from aphids and which served as controls to the infested plants. In all fractions, the numerator represents the total number of plants either infested with aphids or treated as controls, as the case may be; the denominator

the aphids had migrated to the new hosts, the mosaic leaves upon which they had previously been feeding were ground up separately in small quantities of sterile water and the liquid allowed to stand for 48 hours in sterilized test-tubes in order to destroy any cucumber mosaic virus present. The contents of each tube were then inoculated to tobacco to determine the presence of any tobacco mosaic virus in the extract. The experiment was repeated three times and similar results were obtained in each trial (table 4). Of a total of 20 aphid-infested plants, 18 developed typical

TABLE 4.—*Selective virus transmission by Myzus persicae from tobacco plants infected simultaneously with both tobacco and cucumber mosaic*

Tobacco plant to which aphids were transferred from mosaic host[a]	Viruses transmitted by aphids	Results of artificial inoculation to tobacco from aphid-bearing leaf[b]	Selective transmission of cucumber mosaic virus by aphids[c]
No. 1	Cucumber mosaic virus	+	+
2	None	+	−
3	Cucumber mosaic virus	+	+
4	do	−	−
5	do	+	+
6	do	+	+
7	do	+	+
8	do	−	−
9	do	+	+
10	do	+	+
11	do	+	+
12	None	+	−
13	Cucumber mosaic virus	+	+
14	do	+	+
15	do	+	+
16	do	+	+
17	do	+	+
18	do	+	+
19	do	+	+
20	do	+	+

[a] As controls to these aphid-infested plants, 20 tobacco plants were treated with mosaic leaves free from aphids; all remained healthy.

[b] The "aphid-bearing leaf" is the leaf on which the aphids were feeding when transferred from the mosaic host. + denotes infection with tobacco mosaic obtained; − denotes no infection.

[c] + denotes selective transmission demonstrated; − denotes selective transmission not demonstrated.

cucumber mosaic only and 2 remained healthy. None showed any symptoms of tobacco mosaic. Since cucumber mosaic is readily distinguishable from the combination disease, there could be no doubt as to the results secured. All control plants remained healthy. Of the 18 aphid-bearing leaves transferred from the mosaic hosts to these 18 subsequently infected plants, 16 were shown by artificial inoculation to tobacco to contain the tobacco mosaic virus. In 16 out of 20 cases, the occurrence of selective transmission by the aphid was thus definitely established (Fig. 1). It is evident that, although both viruses may be present in the leaves upon which this aphid feeds, the aphid is capable of transmitting only the cucumber mosaic virus from the combination. Similar cases of selective transmission by aphids have been reported, as, for example, that of *Aphis maidis* Fitch, which readily transmits grass mosaic from infected to healthy corn (6), but which apparently does not transmit streak disease to the same host (20).

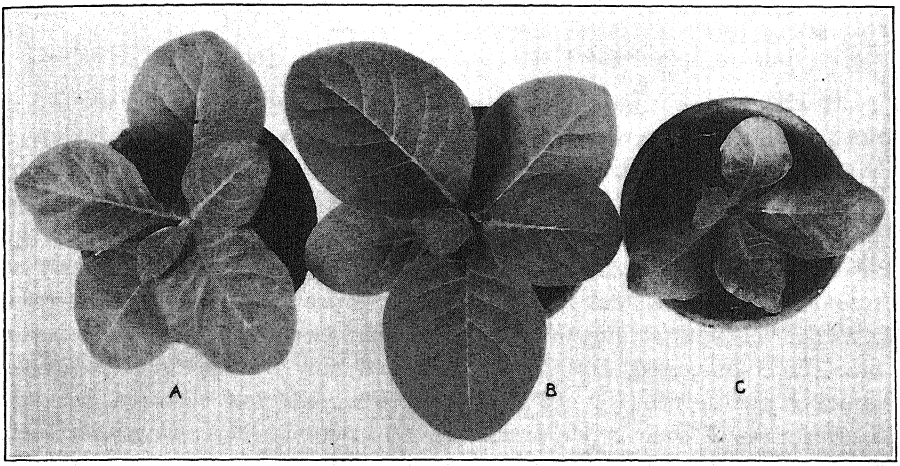


FIG. 1. Illustrating selective virus transmission by *Myzus persicae* Sulz. from tobacco plants infected simultaneously with tobacco and cucumber mosaic.

A. Tobacco plant infested with aphids from tobacco infected with the combination disease. This plant became infected with cucumber mosaic only.

B. Tobacco plant treated as a control to A with a leaf, free from aphids, from tobacco infected with the combination disease. This plant remained healthy.

C. Tobacco plant artificially inoculated with juice extracted from the leaf on which the aphids were feeding when transferred from the mosaic host to plant A. The juice was aged *in vitro* for two days before inoculation. This plant became infected with tobacco mosaic only.

Note the difference in symptoms between cucumber mosaic and tobacco mosaic on tobacco, as illustrated by A and C. In the greenhouse, cucumber mosaic on this host is usually characterized by chlorosis of the whole or part of the leaf, with little or no true mottling or savoying. Tobacco mosaic is usually characterized by a marked stunting of the plant, with distinct mottling and savoying of the leaf.

These results are somewhat surprising, perhaps, in view of the fact that tobacco mosaic is, ordinarily speaking, much the more highly infectious disease of the two among susceptible solanaceous plants. Any slight injury to the epidermis which results in the introduction of a minute quantity of virus into the tissues may be sufficient to cause infection of the plant with tobacco mosaic. The results therefore suggest that there may be something more involved in aphid transmission of viruses than a mere mechanical transfer of viruliferous juice from plant to plant. In this connection it may be appropriate to mention that the peach aphid has been reported as an agent in the transmission of several other plant virus diseases, namely: spinach blight (15), potato mosaic (19), potato leaf roll (5, 18), lettuce mosaic (11), sugar beet mosaic (16), and mosaic of Chinese cabbage, mustard and turnip (17). If these diseases are all due to different specific viruses, then the failure of the peach aphid to transmit the tobacco mosaic virus may have an added significance.

DISCUSSION

It will no doubt be necessary first to correlate the results described in the preceding pages with those reported by Allard (1, 3), who claims to have obtained ready transmission of tobacco mosaic in the greenhouse by the peach aphid. While there is a possibility of this investigator having employed a strain of *Myzus persicae* which differed radically in its behavior in relation to virus transmission, this possibility is considered unlikely on account of the consistent results which have been obtained in the present investigation with strains of this aphid from three widely different localities. It is suggested, however, that Allard may not, in fact, have been securing transmission of ordinary tobacco mosaic, but actually, perhaps, transmission of some other mosaic on tobacco, possibly cucumber mosaic on this host. It seems likely that cucumber mosaic had been introduced at one time or another into his greenhouse. The "specific mosaic disease in *Nicotiana viscosum*" (=*N. glutinosa*), reported by him (2), agrees very closely in description with what we now know as cucumber mosaic on this host, and the symptoms illustrated by him (2, Plate 35) are suggestive of typical infection with this disease. It is possible also that the mosaic diseases of *Phytolacca decandra* (4) and pepper (2), reported by him, may have been cucumber mosaic on these hosts. It should be remembered that, under certain conditions, the symptoms of the two diseases on tobacco may be very similar and difficult to distinguish from one another; that the knowledge of specificity in mosaic disease was at that time relatively limited; and that cucumber mosaic, as such, had not yet been adequately described or recognized in scientific literature. It is therefore tentatively

suggested that, while Allard was essentially correct in his report of the transmission of a virus disease of tobacco by the peach aphid, yet that disease was not ordinary tobacco mosaic, but cucumber mosaic on tobacco.

The results described in this paper have a certain significance in relation to the dissemination and control of ordinary tobacco mosaic in the field. Although all experiments have been conducted in the greenhouse, there seems no obvious reason to expect any radical difference in the behavior of the peach aphid, with respect to virus transmission, under other environmental conditions. Again, while only one form of this insect has been studied, namely, the alate or apterous viviparous female which constitutes the "summer generations," the various other forms apparently do not develop under natural conditions during most, if not all, of the time that tobacco is in the field (10) and on this account would not merit serious consideration from the practical standpoint as possible agents in the dissemination of the disease. While it is probable that certain other species of aphid may be capable of transmitting tobacco mosaic in the field, it appears exceedingly doubtful whether the peach aphid is responsible for the dissemination of this particular disease.

SUMMARY

1. In extensive greenhouse trials, it has been demonstrated that the green peach aphid (*Myzus persicae* Sulz.) readily transmits the cucumber mosaic virus between tobacco and certain other susceptible solanaceous host plants.

2. As compared with methods of artificial inoculation, the peach aphid behaves as a highly efficient agent in the transmission of this virus.

3. Comparative trials, carried out under identical conditions, gave no evidence of transmission of the tobacco mosaic virus by the same aphid between the same host species.

4. The peach aphid exhibits a definite selectivity in virus transmission. Colonies reared upon tobacco plants, infected with both cucumber and tobacco mosaic, transmit only the cucumber mosaic virus from the combination, although the tobacco mosaic virus is present in the leaves upon which the aphids have fed.

5. This selectivity in virus transmission suggests that the relation between aphid and virus may not be purely mechanical.

6. It is not believed that the peach aphid is responsible for the dissemination of ordinary tobacco mosaic in the field.

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EXPLANATION OF PLATES

PLATE I

Illustrating the results secured in comparative trials of the transmission of the cucumber and tobacco mosaic viruses by *Myzus persicae* Sulz.

A. Tobacco infested with aphids from *Physalis pubescens* infected with tobacco mosaic. This plant remained healthy.

C. Tobacco infested with aphids from *P. pubescens* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

B. Tobacco treated as a control to C with a leaf, free from aphids, from *P. pubescens* infected with cucumber mosaic. This plant remained healthy.

D. *Nicotiana rustica* infested with aphids from *N. glauca* infected with tobacco mosaic. This plant remained healthy.

F. *N. rustica* infested with aphids from *N. glauca* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

E. *N. rustica* treated as a control to F with a leaf, free from aphids, from *N. glauca* infected with cucumber mosaic. This plant remained healthy.

G. *Physalis pubescens* infested with aphids from *Nicotiana glauca* infected with tobacco mosaic. This plant remained healthy.

J. *P. pubescens* infested with aphids from *N. glauca* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

H. *P. pubescens* treated as a control to J with a leaf, free from aphids, from *N. glauca* infected with cucumber mosaic. This plant remained healthy.

PLATE II

Illustrating the results secured in comparative trials of the transmission of the cucumber and tobacco mosaic viruses by *Myzus persicae* Sulz.

A. Pepper infested with aphids from tobacco infected with tobacco mosaic. This plant remained healthy.

C. Pepper infested with aphids from tobacco infected with cucumber mosaic. This plant became infected with cucumber mosaic.

B. Pepper treated as a control to C with a leaf, free from aphids, from tobacco infected with cucumber mosaic. This plant remained healthy.

D. *Nicotiana glauca* infested with aphids from *N. rustica* infected with tobacco mosaic. This plant remained healthy.

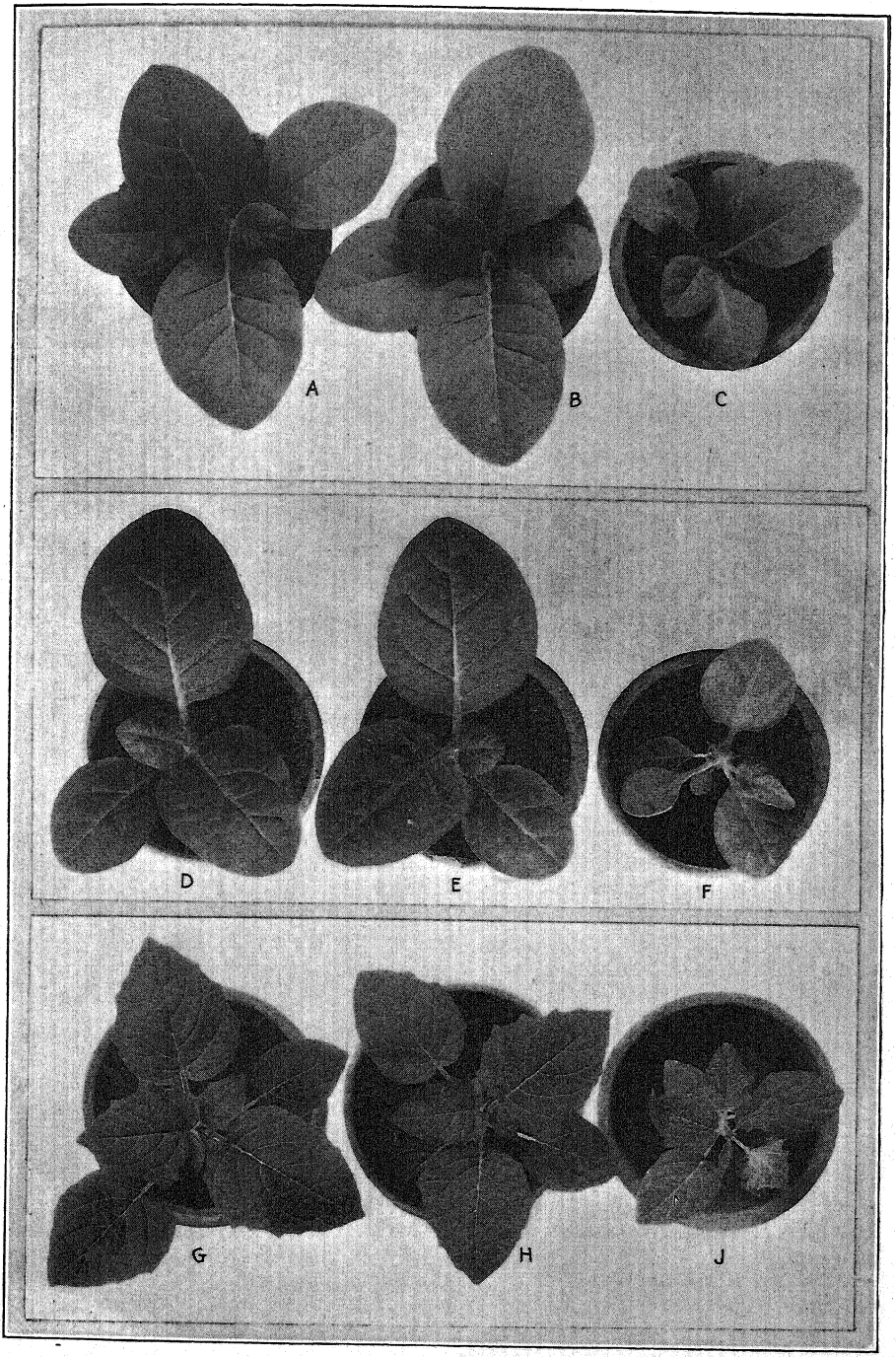
F. *N. glauca* infested with aphids from *N. rustica* infected with cucumber mosaic. This plant became infected with cucumber mosaic.

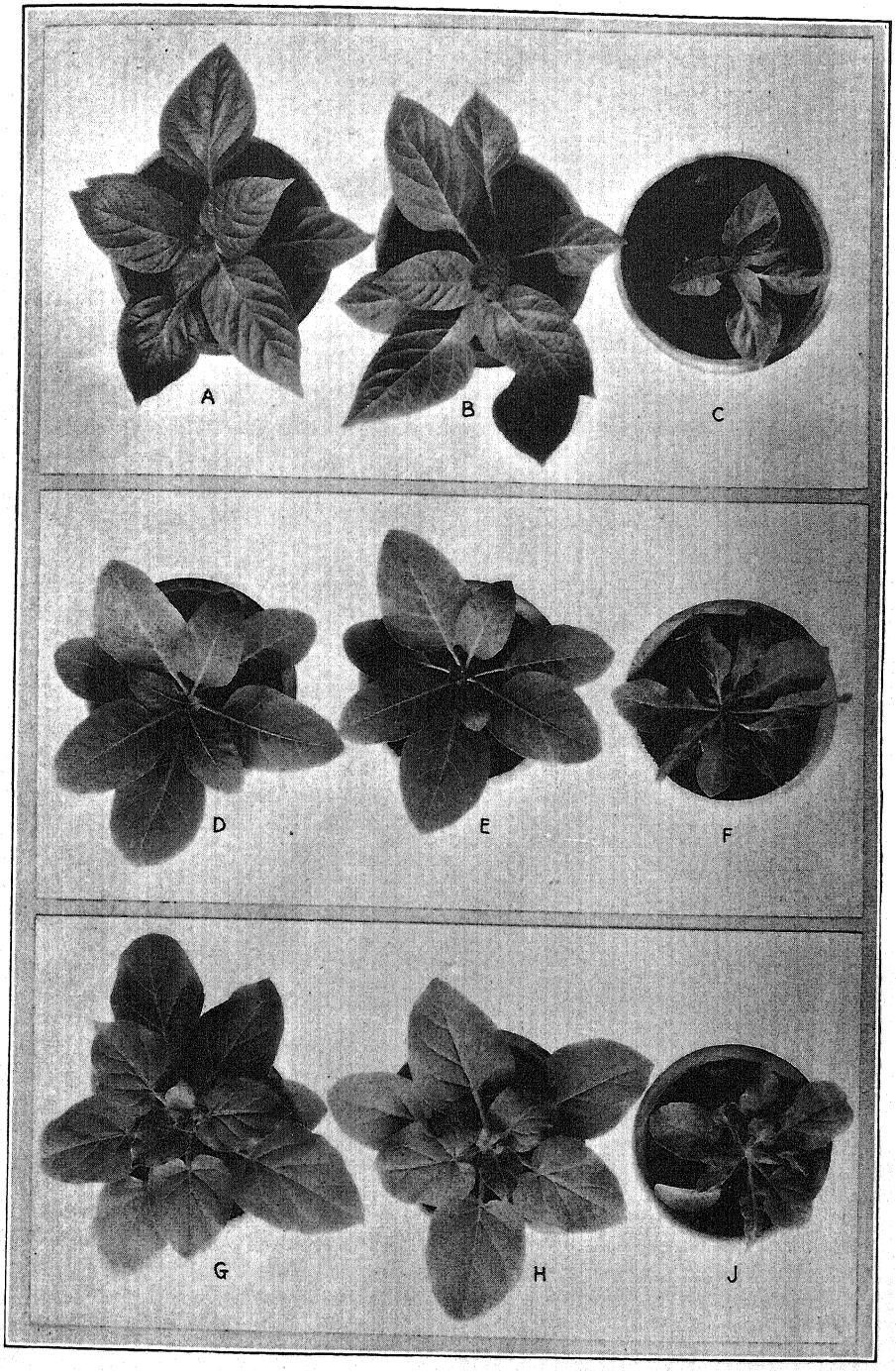
E. *N. glauca* treated as a control to F with a leaf, free from aphids, from *N. rustica* infected with cucumber mosaic. This plant remained healthy.

G. *Nicotiana glutinosa* infested with aphids from pepper infected with tobacco mosaic. This plant remained healthy.

J. *N. glutinosa* infested with aphids from pepper infected with cucumber mosaic. This plant became infected with cucumber mosaic.

H. *N. glutinosa* treated as a control to J with a leaf, free from aphids, from pepper infected with cucumber mosaic. This plant remained healthy.


A
B
C
D
E
F
G
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A
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WHITE-SPOT OF ALFALFA AND ITS RELATION TO IRRIGATION[1]

B. L. RICHARDS[2]

INTRODUCTION

White-spot was first described as a specific disease of alfalfa by Stewart, French, and Wilson (7) in 1908. Since that date various other workers have called attention to the disease, indicating that it is widespread throughout the United States, although of little economic importance. As reported by O'Gara (4) in 1918, the problem under irrigated conditions is somewhat different. Not infrequently in Utah the disease occurs throughout a field and injures the crop.

The factors responsible for white-spot have remained obscure, and in various reports on the disease but little has been contributed to the under-

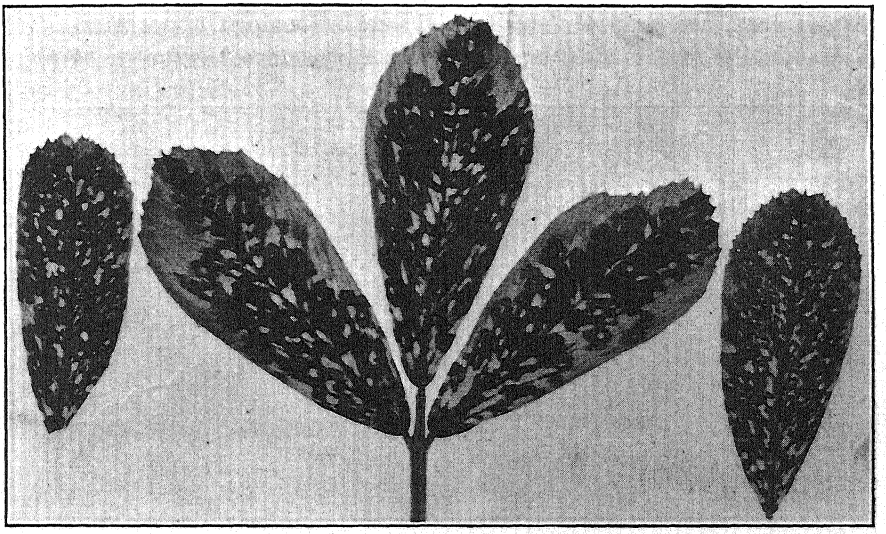


FIG. 1.—White-spot of alfalfa. Leaflets showing spot type lesion, and an entire leaf showing spot and marginal injury.

[1] Contribution from Department of Botany and Plant Pathology, Utah Agricultural Experiment Station. Approved for publication by Director, April 17, 1928.

[2] Botanist and Plant Pathologist. The writer wishes to express his appreciation to Dr. F. B. Wann and H. Loran Blood, of the Botany Department of the Utah Agricultural Experiment Station.

standing of its real nature. In fact, the true cause of the disease as it commonly occurs in the field remains unknown.

In May, 1926, white-spot occurred in such abundance in northern Utah as to cause considerable concern among farmers and to warrant detailed study by the plant pathological division of the Utah Experiment Station. This study was continued through the summers of 1926 and 1927 and has yielded such data with respect to the nature and cause of white-spot as to justify this preliminary report.

SYMPTOMS AND THE IDENTITY OF WHITE-SPOT IN VARIOUS REGIONS

White-spot is characterized generally by writers as small, definitely delimited and bleached necrotic areas in the leaf mesophyll. (See Figs. 1 and 2.) All leaves on a stem, and ordinarily all leaves on all stems of a single crown, become involved simultaneously and usually more or less equally affected. The spots are variable in shape, with abrupt margins. Very little if any spread of the lesion is evident. The clear and uniform appearance of the tissues within the lesions provides little suggestion of a parasitic origin. In fact, the tissues in the early stages of development, though dead, are perfectly sterile. The rather sudden appearance of the disease in the field is striking, and when a considerable number of plants

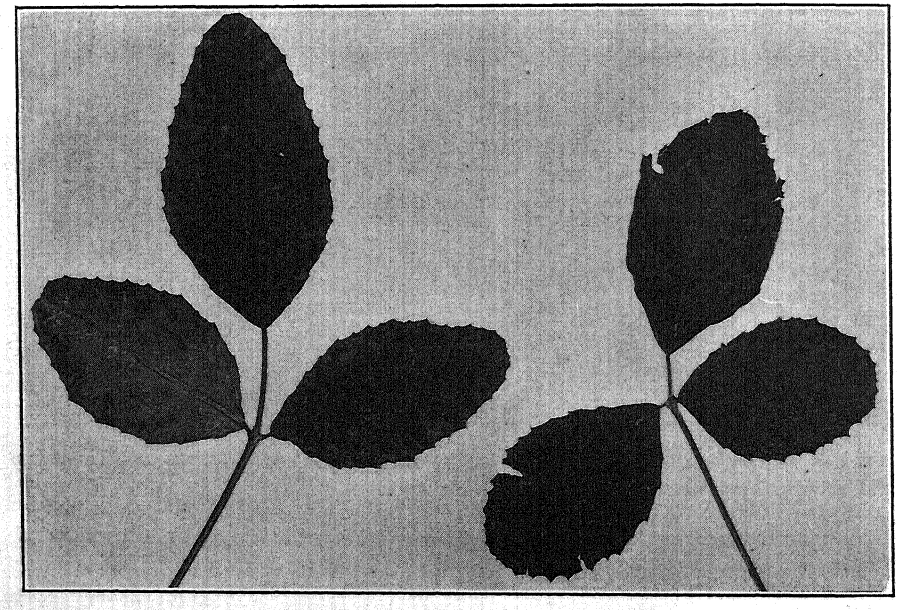


FIG. 2.—White-spot as it appears on sweet clover (*Melilotus alba*). Left, early stage of lesion development. Right, final stage of development.

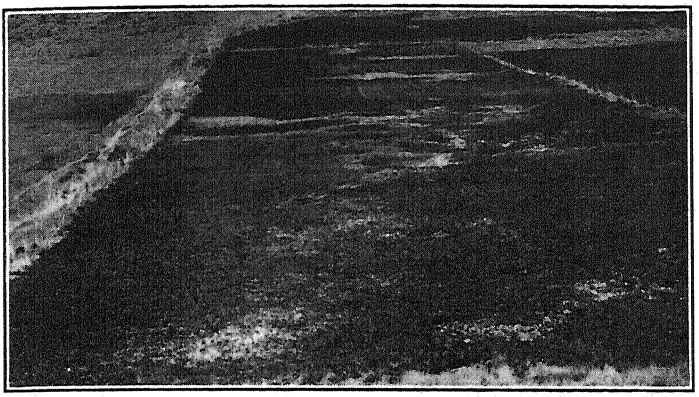


FIG. 3.—Mr. Williamson's field at Farmville in 1926. Irrigated during a heavy rain on May 4, first observed May 9, and photographed May 10. The severely diseased patches, which appear white, developed only in areas of vigorous plant growth. In such patches, 80–100 per cent of the plants were affected.

in an area become affected they may be distinguished from a considerable distance (Figs. 3 and 4).

Since the etiology of white-spot is so elusive, the question naturally arises as to whether the disease as observed by the early workers throughout the United States is the same as that observed so frequently in Utah during 1926 and 1927. Descriptions, photographs, and personal observations, however, confirm rather than question the identity.

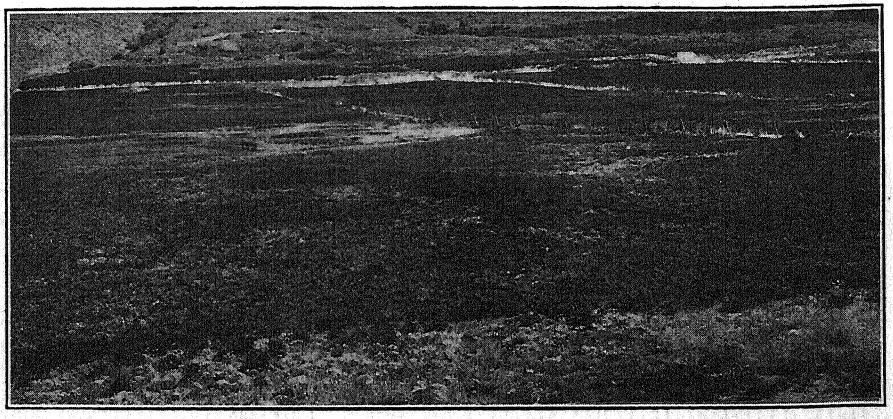


FIG. 4.—Three alfalfa fields in the Farmville district in 1927. At left, Mr. Williamson's field studied in 1926 (Cf. Fig. 3). Water was started on his field at 6 A. M., May 13; on the center field at noon; and on the field at right at 5 P. M. It was allowed to run on all three until 6 A. M., May 14. Photographed May 16, 48 hours later than photograph shown in figure 5.

O'Gara (4) states that he has observed white-spot throughout the Middle Western, Intermountain, and Pacific Coast States; and further states that he has submitted white-spot which was artificially produced in his plots to several plant pathologists, who reported that his specimens were identical with white-spot which they themselves had collected. W. W. Jones (3) identified the disease occurring in 1926 and that produced experimentally by the present writer in the fields of Cache Valley as identical with disease observed previously by O'Gara and him in Utah and other western states. In addition, Jones states (3) that "In the spring of 1917 specimens taken in Salt Lake Valley were sent to F. C. Stewart and C. H. Crabill for comparison with the disease specimens observed in New York and Virginia. Both of these observers affirmed an identity of condition."

During October of 1926 the writer submitted specimens of white-spot produced experimentally in Cache Valley to Dr. F. R. Jones, who recognized the disease as being of the same nature if not identical with that which he had observed in alfalfa fields throughout the United States.

Through the courtesy of Dr. C. G. Coons, in 1926 the writer procured specimens of white-spot collected in Michigan in 1915. Additional fresh specimens were sent from Michigan in 1927 by Professor Ray Nelson. When compared, these specimens from Michigan could not be distinguished from those collected in Utah during the various studies throughout the summers of 1926 and 1927.

Whether or not such peculiar spotting of alfalfa is produced by one or a number of factors, it is quite evident that the lesions appearing in various parts of the United States are so similar as to be indistinguishable.

DISTRIBUTION AND ECONOMIC IMPORTANCE OF WHITE-SPOT

From published reports white-spot is known to occur in New York State (7), Connecticut (1), and Virginia (2, 5), as well as throughout Middle Western, Pacific Coast and Intermountain States (4). In addition, through personal conferences the writer has learned of its occurrence in Michigan, Wisconsin, Illinois, Indiana, and Ohio. It is quite possible that the disease occurs to a limited extent wherever alfalfa is grown.

In general, white-spot is considered of minor economic importance. In speaking of the disease in New York, Stewart (7) states: "The disease occurs in alfalfa fields all over the state, but we have never seen it sufficiently abundant to cause appreciable loss." Clinton (1) observes that the spots are confined to scattered plants in the field and that it is "not a very important or injurious trouble." Similar reports have been given to the writer by various pathologists, although there are others who maintain that the disease is worthy of more than passing attention and that it is

of far greater importance than is usually attributed to it. O'Gara (4) reports that "usually the disease is not considered serious by alfalfa growers, but in many instances the writer has noted that the disease may be more or less disastrous and may produce a very decided loss in yield." A similar view is held by W. W. Jones (3), who has observed the disease in Utah for a number of years.

In the survey conducted during May, 1926, white-spot was discovered in a considerable number of fields in each of the following counties: Salt Lake, Utah, Davis, Weber, Boxelder, and Cache. In Salt Lake, Utah, and Cache Counties the disease was especially severe. For example, in Cache Valley, 98 affected fields were noted along the main road from Wellsville to Richmond, a distance of approximately 20 miles. Several of these fields were seriously damaged, varying in percentage of plants injured from 15 to 75. In the most severely injured fields, a crop loss of as high as 20–35 per cent resulted. This loss consisted in retarded growth, leaf mesophyll destruction, leaf drop, and frequently, in severe cases, the killing of the entire plant.

The following year (1927) the disease appeared in serious form only in local areas, especially in Salt Lake and Cache Counties. In such local areas, because of its annual occurrence, white-spot must be considered as an important factor in alfalfa production.

OBJECTIVES AND METHODS OF STUDY

Studies were undertaken with the prime objective of determining the nature and cause of white-spot, as well as to ascertain more accurately its distribution and its general relation to alfalfa production. Two methods were employed: (*1*) By careful survey it was attempted to discover the peculiar set of conditions under which the disease occurred, and (*2*) by selection of certain fields to produce the disease experimentally under field conditions.

SURVEY STUDIES

The abundance of white-spot in 1926 was first called to the attention of the writer on May 6, two days after the beginning of a heavy rain which fell over the northern part of the state. This was the first rain of consequence which had fallen since April. The various crops, including alfalfa, were suffering heavily as a result of the lack of water. Farmers were beginning to apply the first irrigation water.

On May 7, 8, and 9, fields were studied in the various counties, as follows: Utah, 6; Salt Lake, 10; Cache, 54. A few striking observations will indicate the type of evidence obtained during the survey.

Emil Feulner's Farm, Salt Lake County.—This particular farm was visited on May 7. Irrigation water had flooded part of the west side of the alfalfa patch previous to the visit. The remaining portion had received no water except rain. In the irrigated portion there was white-spot, while none had occurred in the non-irrigated portion.

Joseph Larson's Field, Pleasant Grove, Utah.—In this field, visited on May 7, white-spot areas were conspicuous. Many of the leaves in these areas exhibited incipient lesions, indicating but recent injury. Heavy irrigation had been applied the afternoon and night of May 5, two days prior to the visit.

Fields at Providence, Utah.—Two small alfalfa patches, of approximately 5 acres each, parallel each other. At the time of the visit on May 9 both fields showed serious white-spot injury in an advanced stage. The disease appeared more or less throughout the fields, so that approximately 50 per cent of the plants were affected. In some of the most seriously affected areas 100 per cent of the plants were diseased. The fields had been irrigated on May 4 and 5.

Old Alfalfa Field at Wellsville, Utah.—In a small field of old alfalfa visited on May 13, the disease appeared in an advanced stage on approximately 40 per cent of the plants. It was found that the field had been watered on May 5, one day after the rain.

Fields at Farmville, Utah.—The clearest evidence of the relation of irrigation to white-spot obtained during the survey was found in a study of three small alfalfa fields at Farmville, three and one half miles north of Smithfield, in Cache County. These three fields, of approximately seven acres each, lie adjacent and parallel to each other.

At the time of visit, on May 9, the first field on the north showed heavy white-spot (Fig. 3). White-spot was observed in isolated areas in the center field two days after the first visit but in a less severe form than in the north field (Fig. 4). The south field was entirely free from the disease. Investigation disclosed the fact that the north field had been irrigated heavily on May 4, the day of heavy rainfall; the center field three days later, May 7, two days prior to the first visit; but that the south field had received no irrigation. The south field remained entirely free from white-spot until irrigated on May 27. The following day, May 28, early stages of white-spot lesions were found in abundance, and within 48 hours they had reached their full expression. Other fields with white-spot were visited which showed quite as intimate a relation to irrigation.

During the survey studies careful observations were made as to the state of health of the plants most severely affected. In general, the disease most commonly occurred on plants which were in a vigorous and succulent state

of growth. Plants retarded in growth on account of poor soil or drought occasioned by exposed, gravelly spots in the fields seldom were found affected. This relation was especially clear in the Williamson field (Fig. 3).

No positive correlation was noted between soil type and occurrence of white-spot. The severest form of the disease, however, was found at Farmville, Cache County, on soils derived almost entirely from a dolomitic formation and with a high magnesium content. Again, the disease appeared slightly more abundant in other parts of the state on soils high in calcium. In general, the disease was more abundant and more severe in Cache Valley than in any other part of the state visited.

EXPERIMENTAL STUDIES, 1926

The relation of irrigation to white-spot production was so evident that it appeared entirely possible to produce the disease by the application of irrigation water on fields not previously watered. This was accomplished in abundance during the seasons of 1926 and 1927.

Four fields in Farmville, Utah, were selected for observations. These fields were all examined for the presence or absence of white-spot just prior to the first irrigation. They were then irrigated and subsequently watched to determine the effect of the water on the development of the disease.

On May 14, field No. 1 (8 acres) was carefully examined and found to be entirely free from white-spot. The field was then irrigated during that afternoon, and during the afternoon and night of May 15. The examination was not made until May 20, at which time the writer was confronted with the most severe expression of white-spot he had ever observed. Between 70 and 80 per cent of the alfalfa plants were affected and to a degree varying from but few lesions on the leaves to complete killing of every leaf on all stems of some plants.

In addition, similar lesions were found abundantly on sweet clover (*Melilotus alba*) and occasionally on black medic (*Medicago lupulina*). Upwards of 80 per cent of the sweet clover plants in the areas in which the alfalfa was most severely affected showed lesions. These lesions were comparable to those on the alfalfa in time of occurrence, distribution on the leaf, and in the distribution of the lesioned leaves on the stem. The final color of the lesion on sweet clover was dark brown instead of white or light yellow, as in the case of alfalfa (Fig. 2). The final color expression on the black medic more closely approximated that of the alfalfa lesion.

The second field (No. 2), on which water had run during the night of May 20, was examined in the forenoon of May 21. On this date the very earliest stages of lesion development were found, indicating that the disease developed rather suddenly after irrigation. Full expression of these lesions

resulted in approximately two and one half days from the time of the first observations.

Field No. 3 was examined carefully the evening of May 26, with the result that only one plant within a small area previously flooded was found showing white-spot lesions. Water was allowed to run on part of the field during the next afternoon and evening, May 27. The remaining portion of the field was watered May 28. Examination on the morning of May 29 disclosed a general distribution of white-spot in the early stages of development. Between 25 and 30 per cent of the plants in the field were affected. But a single small area adjacent to a small swamp on which the plants had a constant water supply remained free from the disease. By May 31, the disease had reached its full expression.

Field No. 4, the south field of the three observed at Farmville, was examined on May 27 and found free from white-spot. Water was applied during the night. The early stages of the disease were evident by 9 o'clock the following morning; the disease reached full expression in approximately two days.



FIG. 5.—White-spot affected area in Williamson's field photographed May 14, 1927, at 11:30 A. M., two and one-half hours after the appearance of the first lesion.

EXPERIMENTAL STUDIES IN 1927

In 1927 the studies were confined to five fields in the Farmville district.

On May 10 the writer made his first trip to the Farmville district. At 5:30 P. M., white-spot, in its early stages, was found in a 13-acre field be-

longing to Mr. Hillyard. Water had been turned on this field at 9 A. M., May 9, and had run during the day and part of the night. The first symptom of the disease to be noticed was dropping of the leaves at 3 o'clock the next afternoon (May 10). The field was very severely affected, and the disease caused a loss of approximately one third of the crop. Mr. Hillyard also informed the writer that owing to the severity of white-spot on a 40-acre field in this same district he had been forced to abandon the practice of irrigating the first crop.

During the night of May 10, waste water from the Hillyard farm flooded strips of alfalfa through the north end of the 8-acre field which had been used for experiments in 1926 (field No. 1). Severe white-spot resulted and was confined entirely to the watered area. By request, water was allowed to run during the night of May 11 on additional ground in this same field not previously irrigated. Observations were commenced early the next morning in cooperation with Dr. F. B. Wann and Mr. H. L. Blood of the Department of Botany. At 11:20 A. M., first indications of the disease were noted in the form of opaque pea-green spots in the mesophyll of a few leaves of a single plant. Within 30 minutes similar pea-green opaque lesions appeared over the entire irrigated area. By 2:45 the same afternoon, the disease in this affected portion of the field was distinct enough to be photographed. Bleaching of the lesions was so rapid that by evening of the same day the whitened lesions were clearly noticeable from some little distance. Full expression of the lesions was reached in approximately two



FIG. 6.—Affected spot shown in figure 5, 48 hours after the appearance of the first lesion.

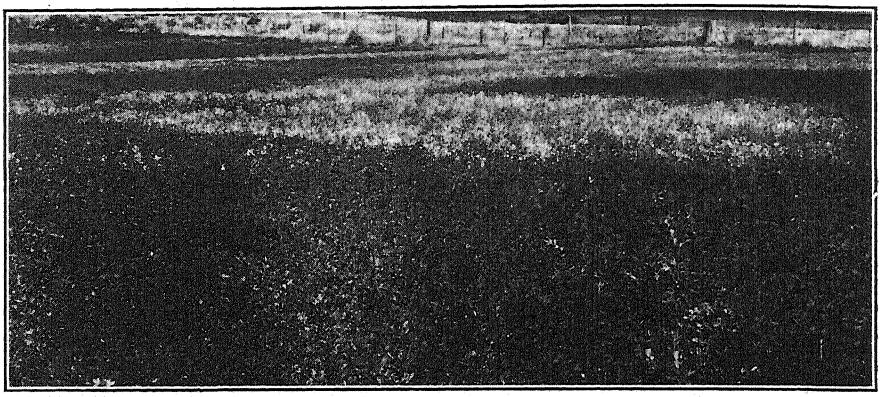


FIG. 7.—Typical white-spot on second-crop alfalfa produced by irrigation, July 4–5, 1927.

and one half days. The remaining portion of this crop was also damaged severely by white-spot from a later irrigation, as in 1926.

At 6 A. M. on May 13, water was started on one division of the field owned by Mr. Williamson.[3] At noon, water was put on a second part of the field, and a third part was irrigated beginning at 5 P. M. The water was allowed to run on all parts during the night and was turned off at 6 A. M., May 14. All three divisions were carefully watched from the time water was turned on the first portion of the field until dark. Obeservations were commenced at daylight on May 14. By 8:30 A. M., the first sign of injury was detected on a single plant in the area watered from 12 (noon) on. Almost simultaneously the lesions were observed on plants in the other two divisions. By 11:30 A. M., the field was photographed, showing distinctly the area affected (Fig. 5). A second photograph was taken on May 16, 48 hours later. (Fig. 6.) Plants watered from 6 P. M. and during the night showed damage as early and of equal severity as those watered for a longer period (Fig. 4).

An adjacent field on the south, rented by Mr. Williamson, the center field of the three studied in 1926, was watered during the night of May 14. Lesions appeared about 9 o'clock the next morning.

At 10 o'clock on the morning of May 15, water was started on one half of the 7-acre field to the south and allowed to run for 10 hours. The water was then turned off and started on the second half, where it was likewise allowed to run for 10 hours (until 6 A. M., May 16). Three hours after the water was turned off lesions were noted on plants in the part irrigated

[3] This was the north field of the three adjacent fields on which observations were made in 1926 and which are recorded in the foregoing discussion.

during the night. In fact, many plants in a number of areas were killed. No white-spot developed on plants watered during the daytime or from 10 in the morning to 8 in the evening, May 15.

Several additional fields were similarly observed after they had been irrigated, with results similar to those detailed. In all cases, lesions were found to develop early in the morning following the irrigation. In no case were lesions found to result during the latter part of the day following daytime watering.

The question naturally arises as to relative sensitiveness of the various crops of alfalfa to possible damage by irrigation. During 1926 the writer noticed slight injury to plants in both second and third crops in the various northern counties surveyed. O'Gara (4) likewise noted the disease on all three crops. In an attempt to study this problem further, the second crop on the 8-acre field (field No. 1, 1926) was irrigated as follows: On Saturday morning July 4, water was turned on the east part of the south half. This ran all day; at night the water was changed to the west division of the south half where it was allowed to run all night. The disease occurred in a very severe form on the part watered during the night. None occurred on that part watered during the day. The north half of the field was watered Monday afternoon and part of the following night, July 4. Lesions appeared abundantly at 8:45 A. M., July 5. The damage on this division of the field was very severe, probably the most severe which had been produced throughout the experiments (Fig. 7). No further tests were made on the second and none on the third crop.

LESION TYPES AND SEQUENCE OF LESION DEVELOPMENT

Descriptions and photographs presented by Clinton (1) and Crabill (2) indicate a peculiar and uniform type of spotting as characteristic of the white-spot disease in New York and Virginia. This same "spot" type of lesion (Fig. 8), likewise characterizes generally the white-spot in Utah, although under certain conditions a very definite marginal injury is found associated with it (Fig. 9). In the first crop, marginal injury may occur on scattered plants in fields where spotting predominates or it may equal in prominence the spot type in the same fields. In 1926, marginal lesions occurred almost exclusively in the second and third crops of alfalfa. In the milder form of the disease marginal injuries may involve but a few cells of the distal portion of the leaf, or under more severe conditions it may involve the entire margin of the leaflet. As with the spot type, in its severest form practically the entire leaflet area becomes affected, leaving but a narrow strip of green along the mid-ribs and a few lateral veins (Figs. 8 and 9). In all cases, as with the spot type of injury, the transition between the diseased area and the healthy portion is very abrupt.

To the writer's knowledge, no worker has described the various stages of lesion development. This phase was carefully observed during the experimental studies. The very earliest expression of the disease appeared in the form of opaque areas in the leaf mesophyll which are especially prominent when the leaf is held between the eye and the source of light. At this early stage no color change is evident. This opaqueness probably results from the substitution of air for water in the injured tissue, the healthy tissue remaining turgid and translucent in appearance. With the substitution of air in the tissues, the lesions sink slightly and rapidly change from deep chlorophyll green to light cress through chrystolite green (6) to a yellowish gray green, and finally with bleaching to white or light yellow or tan. These earlier color changes in the lesions are apparent only on the upper side of the leaf where evaporation is probably most rapid. Later the tissues of the lower side of the leaf go through similar color changes. Finally, within 48–60 hours in dry weather, the affected tissue becomes completely bleached (Fig. 10) and presents a lesion with uniformly smooth and clear surface on both sides of the leaf. On the older leaves, at this time, the affected tissue appears to be dead. In the very young leaflets

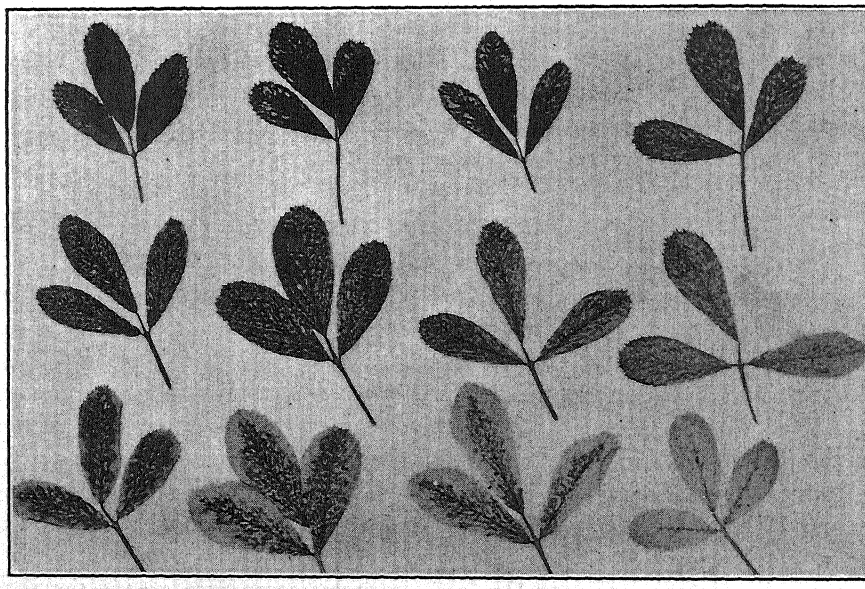


FIG. 8.—Typical white-spot. Beginning with upper left-hand leaf, leaves show gradations from slight injury with few spots to complete whitening of leaf. Veins remain green for some time after bleaching of other parts. This type of lesioning is more common than the marginal type, especially on the first crop, and agrees with white-spot as described by other workers.

at the growing apex, the affected tissue apparently is not killed, and the lesions seldom reach the completely bleached stage. The cells in such affected areas continue to live and in some instances apparently recover their normal green color. Recovery, however, has not been clearly demonstrated. Leaves developed subsequent to the application of irrigation water and the production of the disease are always free from white-spot and are apparently normal.

When the disease develops during rainy weather or during rains subsequent to lesion development, the dead tissue may lose its white appearance and become deep yellow or even dark brown in color. The characteristic color of the lesion is reflected in the appearance of the white-spot area in the field. Fruiting of saprophytes on the dead tissues during rainy periods may add to the color changes.

Injury to the plant may be but slight or the death of the entire plant may result. When injuries are medium to severe, but not resulting in stem destruction, leaf fall may become a serious factor in decreasing the yield and the feeding value of the crop. In a series of counts on a large number

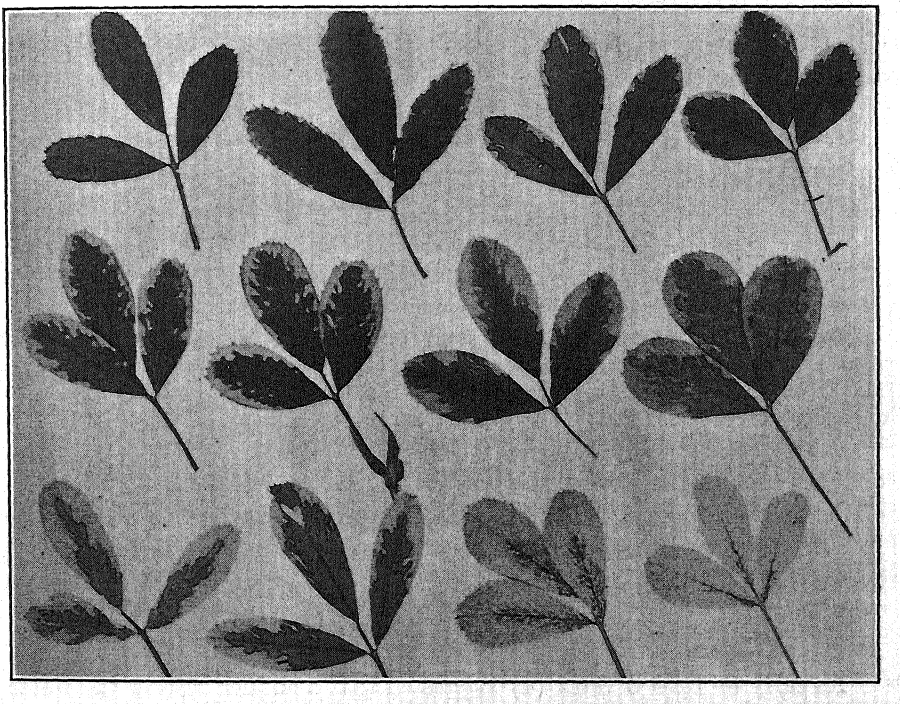


FIG. 9.—Alfalfa leaves showing various degrees of marginal injury produced by irrigation water. The leaves were taken from different plants on the Carbridge 8-acre farm 5 days after irrigation.

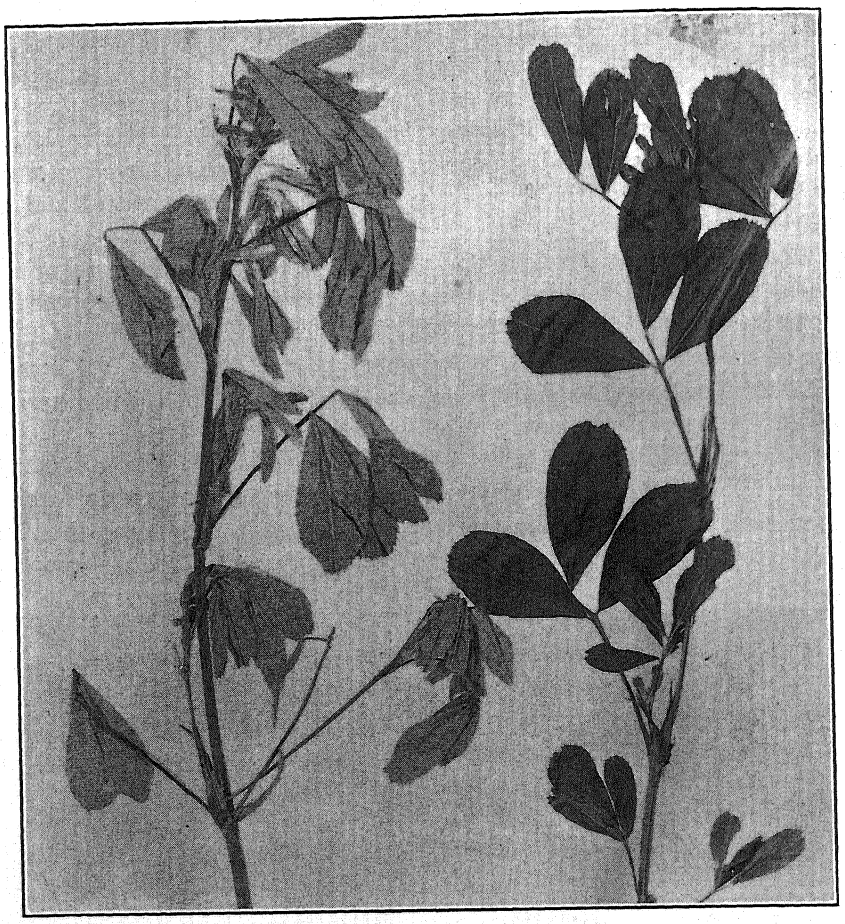


FIG. 10.—Alfalfa stem at left with dead and completely bleached leaves resulting from irrigation. The upper petioles and the younger apical portion of the stem has also succumbed to the disease. Normal stem at right. Both stems were taken from the Carbridge field May 20, 1926, five days after the first irrigation.

of stems about equally affected it was found that an average of 26.5 per cent of the leaves dropped. This leaf fall is especially serious in view of the fact that approximately two thirds of the food value of alfalfa is in the leaves. Farmers report that animals avoid hay affected with white-spot.

DISCUSSION OF RESULTS

As early as 1918 O'Gara (4) suggested that a sudden rise in the water-table in irrigated districts may produce white-spot in alfalfa, and further observed that "irrigated fields showed white-spot trouble, while non-irri-

gated fields showed none." O'Gara describes no specific observations. The experimental results detailed in the foregoing pages, however, leave no doubt as to the accuracy of O'Gara's observations.

Survey data of 1926 suggest that white-spot may result from heavy rainfall, especially when plants are in a state of drought. This conclusion is strongly supported by observation in Michigan. Nelson, of the Michigan Experiment Station, who sent specimens of the disease to the writer, states that the disease was found coincident with a heavy rainfall.

In general, the data indicate that an unbalanced water relation in the soil and subsequently in the plant is an important cause of white-spot and that this condition induced by irrigation is the chief cause of white-spot in the semi-arid West.

From the literature it would appear that factors other than a disturbed water relation are involved in the production of white-spot. Crabill (2) correlates the disease with crown injury and claims to have produced it by artificially mutilating the crown. O'Gara (4) reports the production of white-spot in his experimental plots by watering plants with various solutions. O'Gara does not report details of the experiments: neither does he claim to have checked his experiments by watering plants without substances in solution. In addition, he also correlates the disease with excess alkali. In areas adjacent to smelters white-spot is not infrequently but probably erroneously attributed to injury from sulphur dioxide or other fumes emitted by smelting plants.

It is conceivable that a number of factors may be responsible for white-spot and that mesophyll lesions are a generalized symptom related in a variety of ways to these various etiological factors.

It is significant that injury from irrigation characterized by alfalfa white-spot is not peculiar to this particular plant. It will be recalled that lesions corresponding in nature, time, and sequence of development appeared on both sweet clover and black medic under conditions identical with white-spot lesions of alfalfa (Fig. 2). The lesions on these plants were quite as definitely related to irrigation as were the typical lesions of white-spot on alfalfa plants. It is not unlikely that in white-spot we have a generalized type of injury resulting from seriously unbalanced water relations, common possibly to a large number of plants. With respect to etiology, it is not improbable that such diseases as late blight of sugar-beet, tip burn of lettuce (8), and black heart of celery will fall into this class.

Just how excess water in the soil resulting from heavy rain or the application of irrigation water produces the disease spoken of as white-spot is not clear. Some have suggested that possible absorption of deleterious salts, or salts in excess quantities, which, when left behind in the leaf after

evaporation, kill the mesophyll tissue. A second explanation lies in the possibility that rapid absorption of water may actually burst the mesophyll cells and chloroplasts. A third theory is suggested in the degree to which the sudden filling of the tissues with water resulting from rapid absorption and from the depression of evaporation with increased humidity might decrease the oxygen supply in the plant and thereby interfere with respiration. The by-products of incomplete respiration or inhibited respiration may thus poison and kill the living cells. While it is quite possible that none of these theories offers the correct explanation, the sudden and uniform appearance of the disease, together with its possible relation to the depression of transpiration during the night, seems to support the latter view. On the other hand, the appearance of the disease year after year in the same field suggests the salt-absorption theory. The answer to the question provides an interesting problem for research on which the Department of Plant Pathology of the Utah Agricultural Experiment Station is placing some effort.

SUGGESTIONS FOR CONTROL

To date, experimental studies have not been made with respect to control of white-spot. A few suggestions may be derived, however, from the nature of the disease and the conditions under which it occurs. As a more severe expression of the disease results from excess irrigation following drought, it would seem good practice with the first crop, in areas of annual occurrence of the disease, to water a little earlier in the season, using lighter applications a little more frequently than is commonly the practice. A uniform condition of moisture in the soil appears definitely to lessen the danger from the disease. It is quite possible that in the areas most severely affected by irrigation, the avoidance of night irrigation may aid in preventing the disease. To what extent this latter suggestion will be helpful is not determined. When the disease is so severe as seriously to retard growth of the crop in the affected area, early cutting may be justified in order that the succeeding crop may be given an early start.

SUMMARY

White-spot varies greatly in economic importance throughout the United States where it is known to occur. In certain areas it is of no significance, while in local areas in the West where irrigation is necessary the disease may become a serious factor in alfalfa production.

Affected plants are retarded in growth, have less effective leaf surface, and under severe conditions are killed. Succulent, rapidly growing plants are most severely damaged. Seldom is the stunted and drought-conditioned plant affected. Lesions are of two types: (1) spotting due to localized

killing of the mesophyll tissues between the veins, and (*2*) marginal tissue injury which may affect but a few apical cells of the leaf or may involve the entire leaf margin. Both types may be so severe as to involve the entire leaf surface.

Survey data procured during 1926 showed clearly that white-spot of alfalfa is greatly influenced by rainfall and irrigation. These observations were definitely confirmed by experiments during 1926 and 1927. During these years the disease was produced over a considerable acreage in a number of different fields at different periods and on both the first and second crops. In the various experiments lesions of white-spot were found to occur simultaneously on plants in affected areas in the irrigation fields, usually early in the forenoon after the water had run during the previous night.

Earlier, more frequent, and lighter irrigations are suggested as possible aids in preventing the disease in the first crop.

UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

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PHYSIOLOGIC SPECIALIZATION IN FUSARIUM SPP. CAUSING HEADBLIGHT OF SMALL GRAINS

CHIH TU[1]

INTRODUCTION

The Fusarium headblight (scab) of wheat and other cereals, together with seedling blight caused by similar fungi, is an important factor in cereal growing in many countries of the world. In the United States wheat scab causes heavy damage in the soft winter wheat area and in the spring wheat area. Although the disease has been investigated extensively, no really satisfactory control measures have been devised. The development of resistant varieties apparently is the only adequate method of control. Very little is known, however, about the species of *Fusarium* which cause the disease and about the possible physiologic specialization within the different species. The present investigation was undertaken primarily to determine the relative virulence of the different species of *Fusarium* that cause headblight and to ascertain whether there are physiologic forms differing in pathogenicity and in their relation to environmental conditions. This knowledge is basic to a sound program of breeding disease-resistant varieties.

METHODS AND MATERIALS

Isolations were made primarily from blighted kernels of wheat and oats. The kernels were soaked in 70 per cent alcohol for two or three minutes and then burned over a free flame until slightly charred. The surface-sterilized kernel was then placed in a petri dish containing a suitable medium. Usually a colony of *Fusarium* grew sufficiently large in three or four days to be transferred to test tubes for stock cultures. Stock cultures were stored either in the ice box at 10–12° C. or at room temperature ranging from about 18° to 22° C. All of the experiments were made with monosporous cultures. Sherbakoff's method of single-spore isolation was used exclusively (19, p. 104).

For all inoculation experiments, cultures were grown in Erlenmeyer flasks on a sterile mixture of equal parts of wheat and oat seed. Usually 25 gms. of seed to 50 cc. of water were placed in a 250-cc. flask and auto-

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The writer acknowledges his great indebtedness to Dr. E. C. Stakman, under whom the present work has been done. He also wishes to thank Dr. C. D. Sherbakoff and Dr. H. W. Wollenweber for identifying and supplying cultures of *Fusarium*. Dr. J. J. Christensen also has given valuable assistance.

TABLE 1.—*Sources of the Fusaria of cereals studied*

Fungus	Original host	Place of collection	Date of collection	Previous history
Fusarium graminearum Schwabe form 1	Wheat	Wisconsin	1924	Dr. L. R. Jones' culture, furnished by Dr. Louise Dosdall
do form 2[a]	do	Waseca, Minn.	Aug. 21, 1926	Material furnished by Dr. O. S. Aamodt
do form 3[a]	Oats	Purgatory Springs, Minn.	Aug. 10, 1926	Material furnished by Dr. J. J. Christensen
F. culmorum (W. G. Sm.) Sacc. form 1[b]	Wheat	University Farm, St. Paul, Minn.	July 10, 1926	do
do form 2[b]	do	do	Sept. 16, 1926	do
do form 3[b]	do	Purgatory Springs, Minn.	Sept. 20, 1926	do
F. avenaceum (Fr.) Sacc. form 1	do	Ithaca, N. Y.	Aug. 17, 1926	Material furnished by Dr. E. C. Stakman
do form 2	do	do	do	Culture furnished by Dr. C. D. Sherbakoff
F. solani (Mart. P. Par.) Ap. et. Wr.	do	do	do	do
F. nivale Sor.	Rye	Schlesien, Germany	Summer, 1925	Culture furnished by Dr. H. W. Wollenweber

[a] Cultures identified by Dr. C. D. Sherbakoff.

[b] Cultures identified by Dr. H. W. Wollenweber.

claved for half an hour at 15 lbs. pressure. In making head inoculations, a spore suspension was used, which was made by washing the culture with a definite amount of sterile water and then straining the washing through sterilized cheesecloth.

The seeds of cereals were obtained from Professor A. C. Arny, Division of Agronomy, University of Minnesota. All the seeds used were presoaked in water from 8 to 12 hours and then treated with 0.25 per cent solution of Semesan for 4–5 hours.

The sources of the different Fusaria used in the studies are listed in table 1. Three cultures of *Fusarium graminearum* Schwabe [*Gibberella saubinetii* (Mont.) Sacc.], three of *Fusarium culmorum* (W. G. Sm.) Sacc., and two of *Fusarium avenaceum* (Fr.) Sacc. were found to comprise physiologic forms distinguishable on the basis of their pathogenicity. For convenience these cultures are designated as physiologic forms 1, 2, etc., as in the list. *Fusarium culmorum* form 1 mutated in the course of study and the resulting culture is listed as *F. culmorum* form 1 mutant.

PATHOGENICITY AND PHYSIOLOGIC SPECIALIZATION

The fact that different cereal varieties differ in their degree of susceptibility to Fusarium headblight has long been recognized by investigators of the disease (3, 4, 5, 9, 10, 12, 16, 17, 18, 20). As a result of extensive varietal tests carried on for eight years at University Farm, St. Paul, Minnesota, it was found that the amount of headblight in individual varieties of wheat varied from year to year and in different localities in the same year (9, 10). Christensen and Stakman (9) suggested that "There was also some circumstantial evidence that different parasitic strains may have been present in different years," in order to account for the different results obtained.

In studying the root rot of wheat, Henry (15) found that different strains of *Fusarium moniliforme* Sheldon differed in virulence on seedlings. Recently Greaney and Bailey (14) tested the pathogenicity of 28 cultures of unidentified Fusaria in the greenhouse and found that there were great differences in the degree of virulence on wheat seedlings.

The writer made a comparative study in 1927 of the pathogenicity of six cultures of *Fusarium*, three of *F. culmorum*, and three of *F. graminearum* (*Gibberella saubinetii*). Chiefly the headblight phase of the disease was studied.

In the head-inoculation experiment in the field seven varieties of wheat (Marquis, Minn. 1239; Preston, Minn. 188; Kota, C. I. 5878; Ceres, N. D. 1658; Glyndon Fife, Minn. 163; Pentad, C. I. 3322; Mindum, Minn. 470) and one variety of barley (Minsturdi, Minn. 439) were used as differential

TABLE 2.—*The percentage of headblight, caused by three physiologic forms of Fusarium culmorum and three of Fusarium graminearum in one variety of barley and seven varieties of wheat grown at University Farm, St. Paul, Minnesota, in 1927*

Inoculum	Variety and percentage of head infection							
	Minsturdi barley	Marquis	Preston	Kota	Ceres	Glyndon Fife	Pentad	Mindum
Fusarium culmorum form 1	94.5	5.5	0.0	0.5	0.1	0.0	0.0	1.0
do form 2	61.4	1.1	0.0	0.6	3.9	0.0	3.6	0.0
do form 3	79.5	14.3	17.5	16.2	37.6	4.8	27.3	3.0
F. graminearum form 1	78.6	22.1	23.4	1.6	44.3	6.4	34.9	4.9
do form 2	32.3	0.2	13.2	0.3	0.5	0.0	7.0	1.9
do form 3	18.7	0.0	0.2	0.3	0.2	0.0	1.3	0.5
Check	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

hosts. The eight differential hosts were grown side by side in three 7-foot rows and replicated twice in each plot. The seven plots were systematically distributed in the field in order to reduce the danger of contamination. Each plot was sprayed with a spore suspension of a different culture. The first spraying was done at flowering time and four subsequent sprayings were made at intervals of three or four days, while the check plot was sprayed with sterile water. Spraying was usually done in the evening, when the temperature was relatively low. The results are summarized in table 2.

On the basis of the degree of infection produced by the different cultures, three physiologic forms of *F. culmorum* and three of *F. graminearum* were distinguished. It is interesting to note that headblight in Minsturdi barley ranged from 18.7 per cent when *F. graminearum* form 3 was used as inoculum, to 94.5 per cent when *F. culmorum* form 1 was used. *F. graminearum* form 1 and *F. culmorum* form 3, producing 78.6 and 79.5 per cent headblight respectively, ranked next in virulence on barley. Form 2 of *F. culmorum* and form 2 of *F. graminearum* both were still less virulent on barley. The former produced 61.4 per cent infection, while the latter produced only 32.3 per cent. On wheat the percentage of infection was never so high as on barley. On all varieties of wheat, except Kota, *F. graminearum* form 1 produced consistently higher percentages of hedblight than any of the other cultures. On Kota wheat *F. culmorum* form 3 produced 16.2 per cent headblight as compared with 1.6 per cent produced by *F. graminearum* form 1. *F. culmorum* form 3 was the next most virulent form on wheat.

The rather low percentages of headblight on the wheat varieties inoculated may have been due to the hot, dry weather which followed the flowering period.

In 1928 a similar but more extensive head-inoculation experiment was made. In addition to the cultures used in the previous year, five more were tested, including *F. nivale* (*Calonectria graminicola*), *F. solani,* two physiologic forms of *F. avenaceum,* and *F. culmorum* form 1 mutant. As differential hosts, two varieties of barley (Minsturdi; and Glabron, Minn. 445), seven varieties of wheat (Marquis; Preston; Kota; Ceres; Haynes Bluestem, Minn. 169; Mindum; and Akrona, C. I. 6881) and two varieties of oats (Victory, Minn. 564; and Anthony, Minn. 686) were used. The differential hosts were grown side by side in rod rows in each plot, and the plots were systematically replicated twice. The plants were inoculated in the same manner as those grown the previous year. The results are summarized in table 3.

LE 3.—*The percentage of headblight caused by 11 cultures of Fusarium in 2 varieties of barley, 7 varieties of wheat, and 2 varieties of oats grown at University Farm, St. Paul, Minnesota, in 1928*

Inoculum		Variety and Percentage of Infection										
		Barley		Wheat							Oats	
		Minsturdi	Glabron	Marquis	Preston	Kota	Ceres	Haynes Bluestem	Mindum	Akrona	Victory	Anthony
rium morum	form 1	87.4	89.3	45.5	6.5	42.6	22.4	21.1	48.0	62.3	64.1	62.1
do	form 1 mutant	28.8	56.1	10.6	14.2	4.1	7.6	4.5	18.5	8.0	46.2	43.0
do	form 2	42.4	63.3	29.5	26.2	14.0	28.0	16.6	15.1	27.5	40.1	39.2
do	form 3	85.5	74.8	26.2	41.5	58.1	47.6	47.5	63.5	76.5	70.1	68.2
ena- m	form 1	39.6	47.8	17.0	3.1	8.3	7.6	21.0	27.2	26.5	37.0	51.5
do	form 2	49.0	60.0	31.0	18.2	25.4	31.0	32.5	15.4	16.1	47.2	53.3
ani		39.5	61.6	19.5	10.3	13.5	14.1	17.6	12.2	20.1	27.3	46.1
mi- rum	form 1	84.4	88.8	82.1	23.1	46.0	51.6	24.5	57.3	57.5	49.1	58.2
lo	form 2	40.8	50.7	46.1	6.5	30.5	21.3	15.6	28.5	54.0	32.4	56.2
lo	form 3	32.6	44.1	15.6	13.3	9.1	9.6	8.5	16.0	27.5	78.0	80.2
ale		48.2	62.4	25.1	18.1	29.1	15.1	24.1	9.0	25.3	42.1	54.2
ieck		4.1	5.6	3.2	2.1	3.4	3.0	1.5	2.9	2.0	3.2	4.1

The experiments of 1928 confirmed the results obtained in 1927. From table 3 it is evident that the relative virulence of the physiologic forms both of *F. culmorum* and *F. graminearum* on barley and wheat was practically the same as that in 1927, except that the percentages of infection on wheat were generally higher in 1928 than in 1927. It seems apparent that the relatively successful infection of wheat may have been due to the cloudy and rainy weather following the flowering period in 1928. The ability of the various Fusaria to infect oats varied. *Fusarium graminearum* form 3 was the most virulent form on oats, producing 78.0 per cent on Victory and 80.0 per cent on Anthony, while it was the least virulent on barley and weak on most of the wheats. *Fusarium culmorum* forms 3 and 1 produced nearly as much infection, while *F. graminearum* form 2 was the least virulent on Victory and *F. culmorum* form 2 least virulent on Anthony.

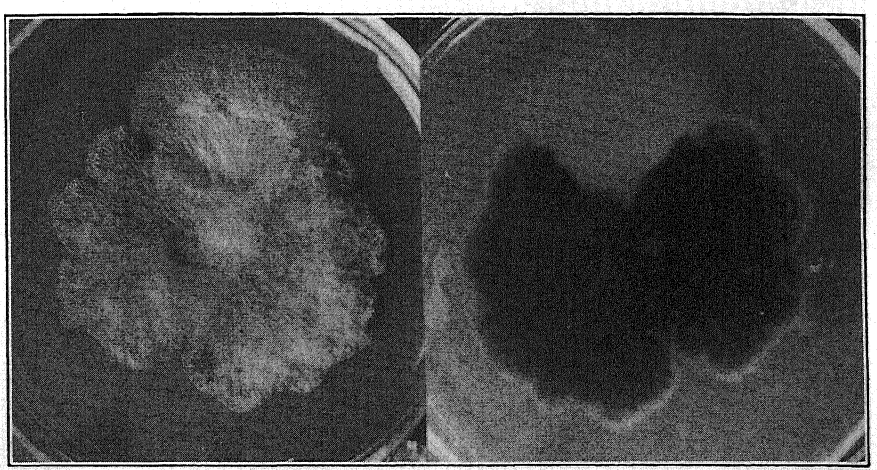


FIG. 1. The mutant of *Fusarium culmorum* form 1. Left: top view; right: bottom view.

The two physiologic forms of *F. avenaceum* differed in their pathogenicity, although they were weak in comparison with other virulent cultures. On barley and oats, and on wheat except the durums, form 2 was consistently more virulent than form 1: the former produced 27.2 per cent infection on Mindum and 26.5 per cent on Akrona, while the latter produced only 15.4 and 16.4 per cent respectively.

Fusarium solani and *F. nivale* were comparatively intermediate in their virulence.

MUTATION

Mutation is a fairly common phenomenon in most of the fungi which have been thoroughly studied in this respect. Appel and Wollenweber (12), in their monograph on the genus *Fusarium,* described certain results which indicated that mutation was taking place, although the authors apparently did not recognize it as such. Later, Sherbakoff (19) probably obtained doubtful cases of mutation in his studies on Fusaria of potatoes. Recently Brown (6, 7) found that "saltation" was a fairly frequent phenomenon in various strains of *Fusarium fructigenum* Fr. on concentrated Richards' solution agar. The present author found, in the course of

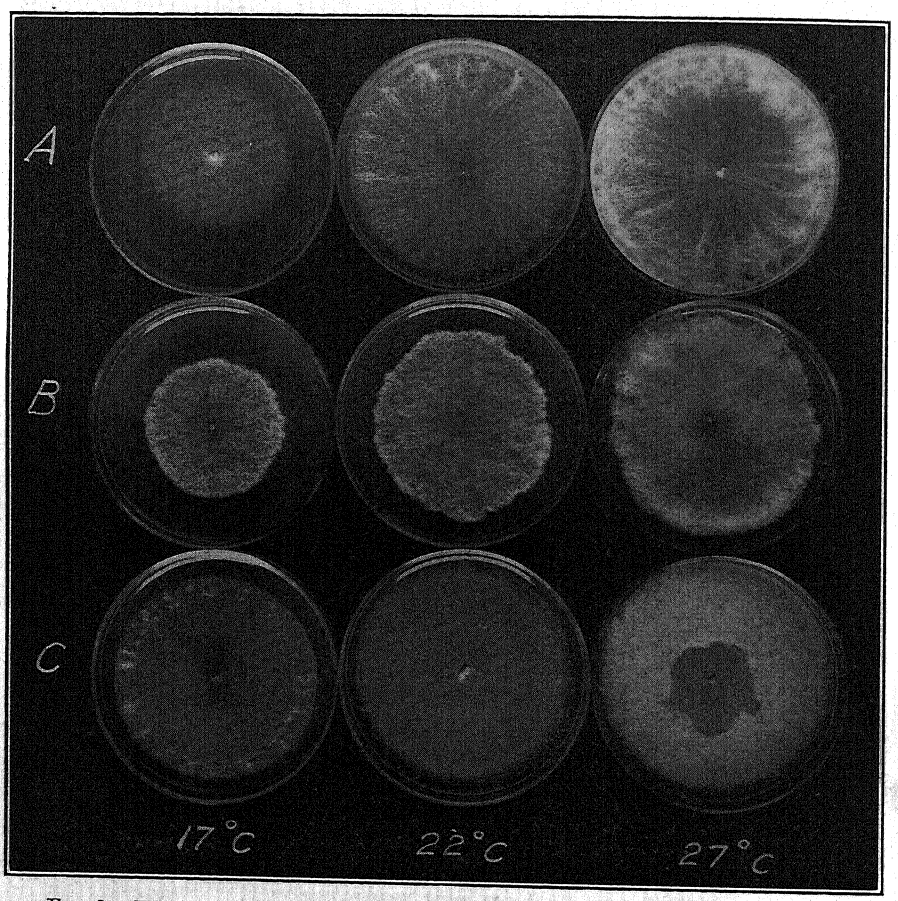


FIG. 2. Effect of temperature on the growth of two physiologic forms of *Fusarium culmorum* and the mutant on Difco potato dextrose agar: (A) *Fusarium culmorum* form 3, (B) *F. culmorum* form 1, (C) mutant of *F. culmorum* form 1. Picture taken 5 days after inoculation.

temperature studies, that *F. culmorum* form 1 mutated at 27° C. (Fig. 1). The mutant differed from the parent in type and rate of growth of the mycelium and in color production at various constant temperatures (Fig. 2). The mutant also produced comparatively fewer spores than the parent, and was much less virulent than the parent in causing headblight of cereals. (See table 3.) However, the factors governing the frequency of mutation on culture media are not very well known at present. Further work along this line is being carried on.

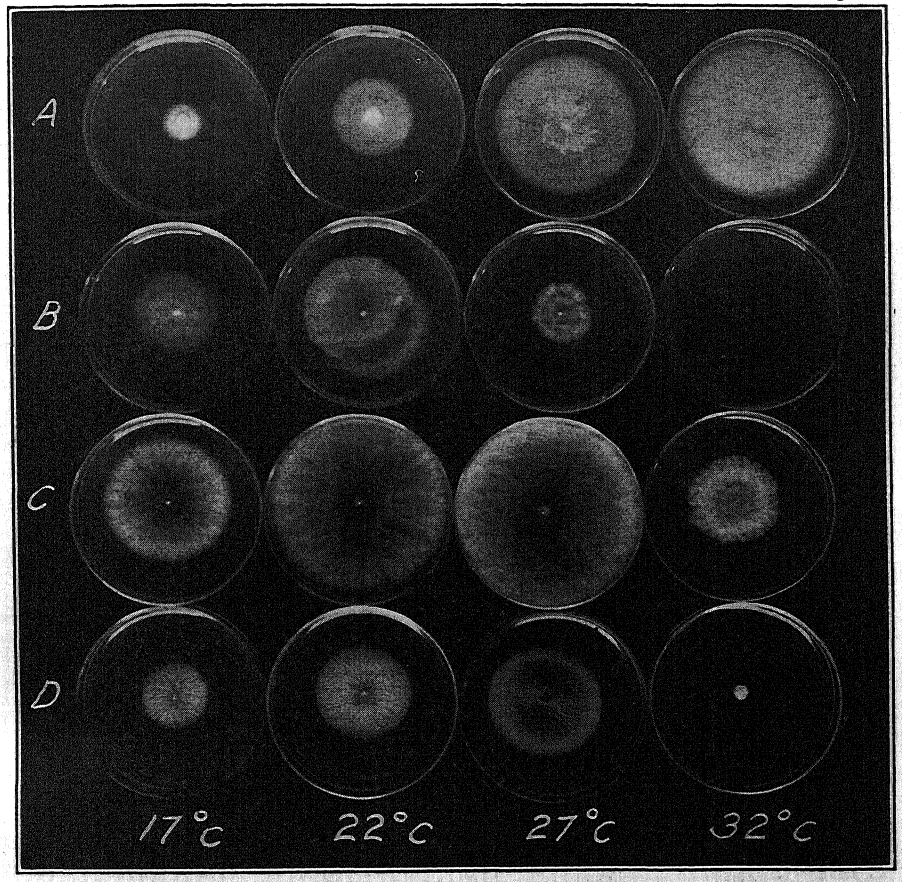


FIG. 3. Effect of temperature on the growth of four species of *Fusarium* on Difco potato dextrose agar: (A) *Fusarium solani*, (B) *Fusarium nivale*, (C) *Fusarium graminearum*, and (D) *Fusarium avenaceum*. Photographed five days after inoculation.

EFFECT OF TEMPERATURE ON GROWTH

The effect of temperature on the mycelial growth of *F. graminearum* was studied by various workers (11, 16). It was found that the organism has the cardinal temperatures of 3°, 25–27°, and 33° C. However, different species of *Fusarium* of cereals may have different temperature requirements, as suspected by Atanasoff (5). Thus a comparative study of the various Fusaria was undertaken by the author. In making the experiment, petri dishes of uniform size were poured with uniform amounts of Difco potato dextrose agar, usually about 18 cc. Bits of mycelium of the cultures to be tested were transferred to the center of the plate. The plates were inoculated in duplicate and were incubated at room temperature for 12 hours before being subjected to the various temperatures. For five days the diameter of each colony was measured daily. The averages of the three experiments for the fifth day are summarized in table 4.

TABLE 4.—*Effect of temperature on the growth of various Fusaria*

Fungus		Temperature in degrees C.					
		7	12	17	22	27	32
		Growth in millimeters 5 days after inoculation					
Fusarium culmorum	form 1	14.5	35.0	45.1	57.3	64.5	34.0
do	form 1 mutant	11.0	34.7	47.2	84.5	86.5	32.5
do	form 2	15.0	42.1	45.7	59.4	68.3	32.5
do	form 3	14.2	40.3	59.5	84.1	89.9	22.3
F. avenaceum	form 1	13.2	35.7	45.6	60.5	42.3	14.1
do	form 2	12.0	23.5	28.7	40.8	49.9	19.5
F. solani		8.1	10.6	18.5	34.2	64.7	71.8
F. graminearum	form 1	15.2	37.0	55.3	83.1	89.2	41.5
do	form 2	15.4	25.6	42.3	89.1	98.9	35.7
do	form 3	12.5	35.2	40.5	52.8	67.5	23.1
F. nivale		14.3	31.4	34.7	48.5	25.3	.....

It was found that some of the Fusaria had different temperature requirements, although most of them were similar to *F. graminearum* in this respect. Two interesting cases were *F. solani* and *F. nivale*. The optimum temperature of the former was about 32° C., while the latter grew best at

about 22° C. and did not grow at all at 32° C. (See figure 3.) It was also interesting to note that the two forms of *F. avenaceum* differed in their temperature requirements: form 1 grew best at 22° C., while form 2 grew best at 27° C., as most of the Fusaria did.

DISCUSSION AND CONCLUSIONS

As a result of the preliminary study of 10 cultures of Fusaria of cereals, experimental evidence proves that there are at least 3 physiologic forms of *Fusarium culmorum,* 3 of *Fusarium graminearum,* and 2 of *Fusarium avenaceum,* which are differentiated by their pathogenicity on different varieties of cereals. There also were differences in pathogenicity among the different species of *Fusarium* studied. Further, it was shown that different Fusaria may have different optimum temperatures for growth. From the results thus far obtained it is quite possible that different physiologic forms, or even different species of *Fusarium,* might predominate in different years on account of variation in weather conditions. If such were the case, the confusing results in breeding work encountered at present might be explained to some extent. Of course, the effect of environment on the development of the host plant and its resistance to Fusarium attack probably is important also.

The occurrence of mutation in *Fusarium culmorum* form 1 may complicate the problem further, for it may alter the pathogenicity of the organism considerably.

SUMMARY

1. A preliminary, comparative study of 10 different Fusaria of cereals was made.

2. Experimental evidence proves that there are three physiologic forms of *Fusarium graminearum* (*Gibberella saubinetii*), 3 of *Fusarium culmorum,* and 2 of *F. avenaceum* which can be differentiated by their parasitism on varieties of cereals. The two physiologic forms of *F. avenaceum* also have different temperature requirements.

3. There also were differences in pathogenicity in the five species of *Fusarium* studied.

4. Mutation occurs in *Fusarium culmorum* form 1 on culture media.

5. The optimum temperature for growth of *Fusarium avenaceum* form 2, three physiologic forms of *Fusarium graminearum* (*Gibberella saubinetii*), and three of *Fusarium culmorum* and its mutant on Difco potato dextrose agar is about 27° C. *Fusarium avenaceum* form 1 and *Fusarium nivale* (*Calonectria graminicola*) have an optimum of about 22° C., and *Fusarium solani* an optimum of about 32° C.

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THE INFLUENCE OF TEMPERATURE ON THE FREQUENCY OF MUTATION IN HELMINTHOSPORIUM SATIVUM

J. J. CHRISTENSEN[1]

INTRODUCTION

Recently the effect of environmental factors on mutation has received considerable attention. There is good evidence that the frequency of mutation in fungi can be stimulated in various ways. According to Brierley (1), Arcichovskij, Waterman, and Schiemann claim to have induced mutation in *Aspergillus niger* and *Penicillium glaucum* by the addition to the culture medium of such chemicals as zinc sulphate and potassium bichromate and by the exposure of cultures to high temperatures. Brierley (1) repeated much of their work but was unable to confirm their results. Christensen (3 and 4) has shown that a given form of *Helminthosporium sativum* may mutate more frequently on one nutrient medium than on another. He also found that some of the physiologic forms mutated readily, while others did not. Brown (2) concluded that a medium which combines high nutrient concentration with minimal staling capacity was conducive to mutation in Fusaria. It also has been demonstrated (3 and 4) that the amount of medium affects the frequency of mutation in *H. sativum,* frequency being greatest when small amounts are used.

In preliminary experiments the writer obtained circumstantial evidence that temperature affected the frequency of mutation in *H. sativum.* A detailed study therefore was undertaken to determine definitely the effect of temperature on mutation in this species.

METHODS

All of the parasitic races of *H. sativum* used in these experiments were monosporous cultures; and in some cases the cultures were obtained by a series of successive single-spore isolations. The numbers used to designate the forms correspond to those listed in table 5 of the author's earlier publication (4).

[1] Published with the approval of Director as paper No. 811, of the Journal Series of the Minnesota Agricultural Experiment Station.

The writer wishes to express his appreciation to Dr. E. C. Stakman for help and criticism in preparing this paper.

The culture medium consisted of 18 gms. of Difco potato dextrose agar and 10 gms. of Difco cornmeal agar in 1 liter of distilled water. The medium for each experiment was made up in one lot, tubed and sterilized. All petri dishes were poured at the same time. In each experiment triplicate series of standard, 90-mm. petri dishes, each containing 18 cc. of the medium, were inoculated in the usual manner with the individual forms. After inoculation, the plates were kept at room temperature for one day and then placed in their respective incubators.

Two experiments were made: the second, one year later than the first. These duplicate series were incubated in two different buildings to eliminate in as far as possible the effect of gases, variation in humidity, and other factors which might possibly stimulate mutation.

RESULTS

The first test was made in March, 1927, with six physiologic forms: 1, 9, 10, 12, 22, and 35. Triplicate plate cultures of each form were incubated at each of the following temperatures: 3°, 9°, 15°, 20°, 25°, 27°, 30°, and 35° C. Twelve days after inoculation the number of sectors in each set of three cultures was counted. After the readings were completed

TABLE 1.—*The number of sectors produced by six physiologic forms of Helminthosporium sativum incubated at eight different temperatures, March, 1927*

Temperature, degrees C.	Physiologic forms						Total no. sectors at each temperature
	1	9	10	12	22	35	
	Number of sectors[a]						
3	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0
20	0	0	1	0	0	0	1
25	19	35	16	0	21	13	104
27	8	49	10	2	9	4	82
30	0	0	0	21	0	0	21
35	0	0	0	0	0	0	0
Total no. sectors in each form	27	84	27	23	30	17	208

[a] Total number of sectors produced in three petri dish cultures.

the cultures kept at the lower temperatures were returned to their respective chambers and incubated until their radial growth was approximately the same as the growth of cultures in which the greatest number of mutations occurred. The additional incubation period lasted from one to three weeks, depending on the temperature at which the cultures were kept, but those kept at 3° C. never achieved the radial growth which the other cultures did. Readings were again made, but in no case was there an increase in the number of sectors over that in the original count. The results obtained are presented in table 1 and figure 1. In each case the number represents the total number of sectors, or apparent mutations, which appeared in a set of three plate cultures.

It is obvious from table 1 that temperature has a profound effect on the frequency of mutation. Thus in the 18 colonies kept at 25° C. there de-

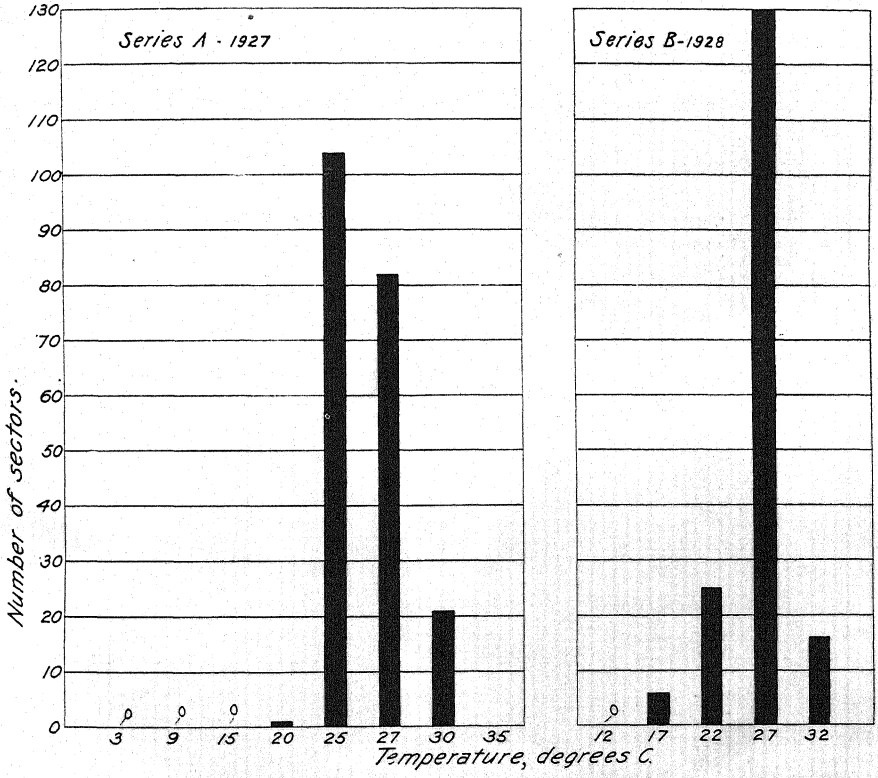


FIG. 1. Graphic representation of the effect of temperature on the frequency of mutation in *Helminthosporium sativum* growing on cornmeal-potato-dextrose agar. Series A and B represent the total number of sectors produced by six and four physiologic forms, respectively.

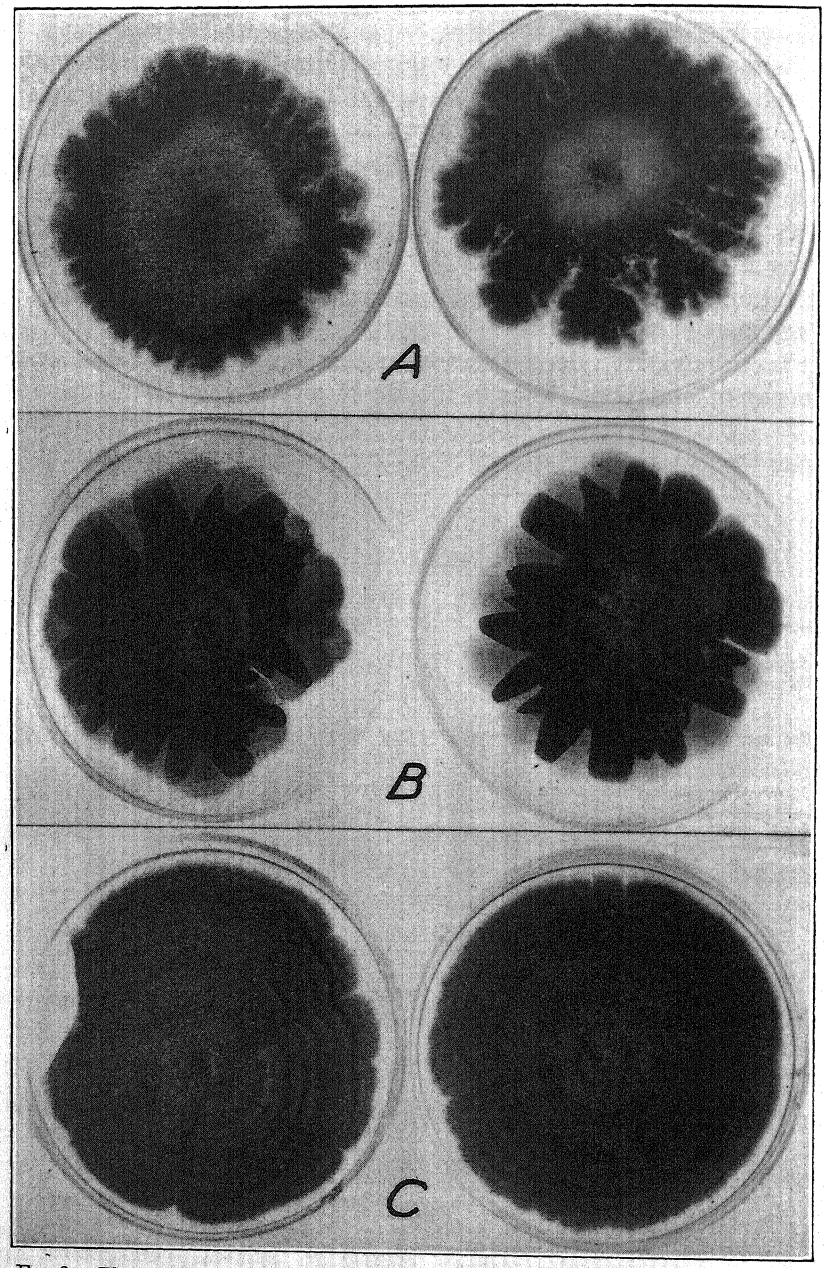


FIG. 2. The effect of temperature on the frequency of mutation in *Helminthosporium sativum*, form 9, growing on cornmeal-potato-dextrose agar, March, 1927. A, B, and C show cultures grown at 15°, 27°, and 30° C., respectively. Note the numerous sectors shown in B.

veloped 104 sectors; in the 18 colonies at 27° C., 82; in those at 30° C., 21; and in those at 20° C. only 1. No sectors were produced at the lower temperatures, 3°, 9°, and 15° C., or at the highest temperature, 35° C. (See figure 2.) At 3° and 9° C. the colonies grew very slowly during the entire experiment, but at 35° C. they grew slowly at first and then stopped growing after a few days.

In April, 1928, a similar test was made, but only four of the six forms previously used were included. Cultures were incubated at the following temperatures: 12°, 17°, 22°, 27°, and 32° C. The data are given in table 2 and graphically presented in figure 1. The results obtained in 1928

TABLE 2.—*The number of sectors produced by four physiologic forms of Helminthosporium sativum incubated at five different temperatures, April, 1928*

Temperature, degrees C.	Physiologic forms				Total no sectors at each temperature
	1	9	10	22	
	Number of sectors				
12	0	0	0	0	0
17	1	0	3	2	6
22	3	2	13	7	25
27	26	43	28	33	130
32	0	12[a]	4	0	16
Total no. sectors in each form	30	57	48	42	177

[a] Nine of these mutants occurred in one colony, the development of which was arrested by some unknown factor.

agreed with those obtained in 1927, although the range of temperatures at which mutation occurred was somewhat wider in 1928 than in 1927. In 1928 sectors appeared in cultures kept at 17° C. and at 32° C.

This experiment again tended to prove that temperature exerts a tremendous influence on frequency of mutation. The optimum temperature for mutation in the 1928 series was 27° C. Unfortunately in 1928 no cultures were incubated at 25° C., which was the optimum temperature for mutation in the 1927 series.

The question naturally arises as to whether these sectors are true mutations or only modifications—temporary phenotypes—induced by environmental factors. The writer (3 and 4) has previously presented evidence that such sectors, at least in many cases, were the result of real genotypic

changes. Since that time more evidence has been obtained in support of this conclusion. A number of mutants of *H. sativum*, together with their respective parents, have been cultured for from one to four years under various conditions. Parents and their mutants have been exposed to conditions as nearly identical as possible. Nearly all of the mutants are to-day as distinct in cultural characteristics as they were on their first transfer (Fig. 3). Two of the mutants now appear to be identical with the parent

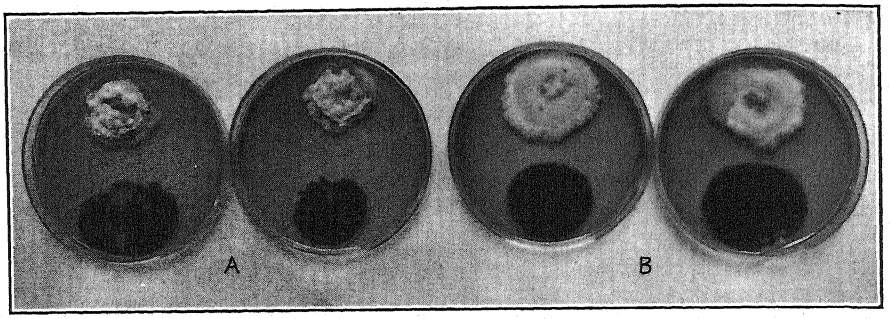


FIG. 3. Parents and respective mutants growing in the same plates on 1 per cent glycerin-potato-dextrose agar, three and one half years after isolation of the mutant. A. Bottom: parent, form 22. Top: mutant, M–40. B. Bottom: parent, form 1. Top: mutant, M–1.

strain, but this is to be expected, as reversion occurs rather commonly, usually in the form of sectors (5).

In April, 1928, the virulence of three mutants, M–1, M–40, and M–5x, was compared with that of their respective parents. The tests were similar to those previously described (4). Mutants M–1 and M–5x possessed the same relative parasitic capabilities as they did in tests made three years previously. The virulence of the mutant M–40, however, had changed considerably. In 1925 this mutant was decidedly more virulent than its parent (4). In 1928, however, it was less virulent than its parent, form 22: in fact it was almost innocuous.

In order to test further the stability of mutants, wheat was inoculated with five of them. On reisolation four of the mutants remained unchanged in cultural characteristics and one, M–40, appeared to be slightly different after reisolation (Fig. 4). The change in this one, however, was not toward the parental characteristics. Three of these reisolated mutants were then compared as to their stability in virulence. Their sojourn on the host did not change their pathogenicity. Even the mutant M–40, which is but weakly parasitic, remained unchanged. There is no good reason of course why mutants of *H. sativum* should not mutate further in their parasitic

capabilities during their stay on living hosts. While the results in this case were negative, positive results might have been obtained if the experiment had been performed on a larger scale. These tests, however, substantiate the fact that the sectors induced by temperature are not mere ecads but true mutants.

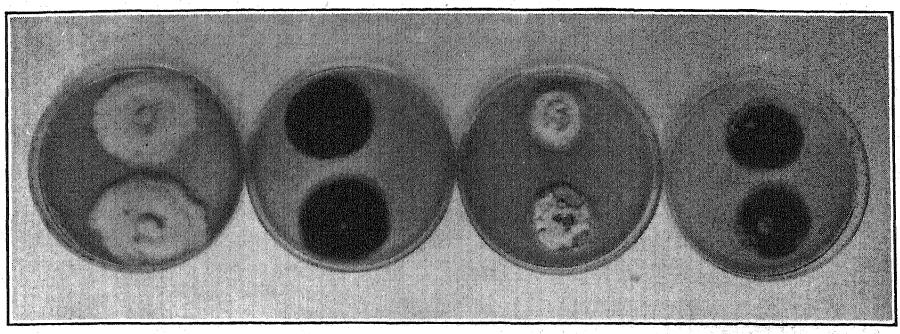


FIG. 4. Effect of passing mutants through a living host. Colonies in top row were obtained from culture reisolated from the host in April, 1928. Those in the bottom row are transfers from the original cultures. From left to right: mutants M–1, M–5xd, M–40, and M–s–27.

DISCUSSION AND CONCLUSIONS

The present studies have shown that temperature has a profound influence on the frequency of mutation in *Helminthosporium sativum* on culture media. The results indicate that moderately high temperatures induce mutation. By far the greatest number of mutants appeared at temperatures between 25° and 30° C. There was some indication that the optimum temperature for mutation in forms 9 and 12 was slightly higher than the optimum for mutation in forms 1, 10, 22, and 35. However, more work is necessary before a definite statement can be made on this point. Mutations seldom occurred at temperatures below 20° C. or higher than 30° C. Although mutations were not observed at the lower and relatively high temperatures, it is possible, but not very probable, that they did occur in masked form. It is obvious that in the study of mutation in fungi one must take into consideration the effect of temperature on the frequency of this phenomenon.

During the past four years the writer has observed hundreds of sectors, or apparent mutants. Many of them have been isolated, cultured, and studied through many clonal generations. Several of these mutants have remained strikingly different from their parents for nearly four years, but some have reverted to the parental type. In general, growing mutants on the living hosts had no apparent effect on the stability of the mutants.

The cultural characteristics of all the mutants which were passed through the living host, with one exception, remained unchanged. Their pathogenic capabilities remained stable in all these tests. These facts support the view that sectors usually are true mutants and not mere phenotypic changes.

SUMMARY

1. A study was made, under controlled conditions, of the effect of temperature on the frequency of mutation in *Helminthosporium sativum* grown on artificial media.
2. Temperature has a profound effect on the frequency of mutation. The optimum temperature for mutation in *H. sativum* lies in the vicinity of 25° to 27° C. No mutation occurred in cultures grown at 15° C., or temperatures lower than this. Comparatively speaking, only a few mutants developed in cultures kept at temperatures below 25° C., or higher than 30° C.
3. Some of the mutants of *H. sativum* are as distinct to-day as they were on the date of isolation, nearly four years ago. Some reversions have occurred.
4. The passage of mutants through living hosts, with one exception, did not appear to change the cultural characteristics of the mutants. Their pathogenicity remained constant.

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BROOMING DIEASE OF BLACK LOCUST (ROBINIA PSEUDACACIA)

CARL HARTLEY AND FERDINAND W. HAASIS

Tree diseases of the mosaic or filterable-virus type appear to be few. Among the forest trees, spike disease of sandal is the only well-known case. When as much search has been made for such diseases in the forests as has been made in the fields, we may find plenty of them. It seems worth while to put on record a disorder of black locust suggesting the virus diseases, which has been known to botanists and foresters for many years but which appears to have escaped mention in the literature.



FIG. 1.—Terminal witches'-brooms on root sprouts of black locust, Asheville, N. C., September, 1926.

The characteristic witches'-brooms are shown in figure 1. There are proliferation and reduction of both shoots and leaves, and the brooms are always erect or ascending. On a single tree 7 inches in diameter at breast height, whose crown was perhaps two-thirds broomed, cushions were found on the trunk. The typical cushion is 4–5 cm. in diameter and approximately 1 cm. thick. The cushion bears groups of buds and tiny leaves on its surface, and is regarded as merely an extreme form of broom.

A number of broomed sprouts were observed and tagged on a permanent sample plot established by the Appalachian Forest Experiment Station on Curtis Creek, North Carolina, in the spring of 1922. All were dead when the plots were examined the following year. On older trees branches which bear brooms die back from the tips; the brooms themselves are also short-lived.

The earliest collections in the possession of the Office of Forest Pathology of the United States Department of Agriculture for the states indicated are as follows:

Roanoke, Virginia, August, 1908; by Horace Engler.
Chevy Chase Lake, Maryland, September 15, 1908; by Dr. W. A. Taylor.
Elijay, Georgia, August 10, 1914; by Dr. G. G. Hedgcock.
Asheville, North Carolina, August 9, 1927; by R. M. Nelson.
Mont Alto, Pennsylvania, January, 1928; by Carl Hartley.

Dr. Erwin F. Smith and Dr. Haven Metcalf independently observed the disease near Washington, D. C., some years ago, and Dr. M. B. Waite has had the disease under observation in Virginia and on his farm in Maryland since 1899 (oral communications). A number of foresters, including Professor L. J. Young and Professor E. A. Ziegler, are familiar with the disease in different parts of the Appalachians.

The disease is frequent on sprouts arising from the roots and stumps of trees that have been cut. In some cases the entire sprout is a broom, making a low, tiny-leaved plant which the uninitiated would never suspect of relationship to locust (Fig. 2). More often the brooms develop terminally or in axils of normal leaves near the top of the sprout. In at least one case, observed by Mr. G. F. Gravatt, the parent tree had appeared healthy at the time of cutting. Among larger trees the disease is apparently rare, but the frequent appearance on sprouts following cutting operations makes it seem possible that many of the older trees are infected without showing recognizable symptoms. The 7-inch tree with brooms and trunk cushions already referred to, and two other trees about 13 inches in diameter, with parts of their crowns broomed but without cushions, were found in the Chevy Chase district near Washington, D. C. The brooms are so numerous on trees that show any of them that they are obviously due to some systemic

factor rather than to local infections. Were the disease entirely limited to sprouts, it might be attributed to abnormal physiological conditions, but that seems impossible in the case of the larger affected trees which in environment and history appear identical with their normal neighbors. The

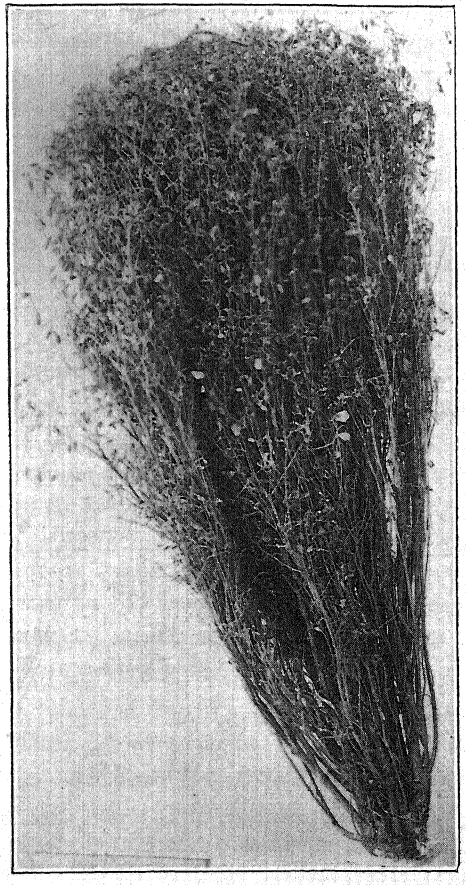


FIG. 2.—Witches'-broom of black locust arising from root of girdled tree, Woodwardville, Md., October, 1921. ×7/16. By courtesy of Dr. M. B. Waite.

Office of Forest Insects, United States Department of Agriculture, has had the disease under observation and finds no reason to suspect an insect cause, though aphids are numerous on cushions and brooms. A further study of the disease is being made by Lyle W. R. Jackson, of the Office of Forest Pathology. It is within the range of possibilities that both quantity and quality of wood produced by many apparently normal trees, and perhaps

even such characters as attractiveness to the locust borer, may be influenced by masked infections. In other parts of the world where black locust is extensively planted, it is suggested that precautions be taken against the introduction of this disease, which under other conditions might cause more damage than it does in the United States.

Systemic witches'-broom diseases are known in several other genera of legumes. Reports under the names rosette, bunching disease, and Kräuselkrankheit, indicate much damage to peanuts from disease of this type in different parts of Africa.[1] The similar krulziekte affects the same crop in Java.[2] The pale dwarf disease of peanut[3] results in plants which superficially resemble the smallest of the completely broomed locust sprouts, but are essentially different in that they show a mere reduction of parts. The leaves are small and closely bunched in the case of pale dwarf, but there is no other change in habit and no proliferation, the leaves being no more numerous than on healthy plants of equal age.

Rutgers, in his aforementioned krulziekte paper also figures an apparently systemic brooming disease of *Phaseolus mungo* in Java, and notes that similar phenomena have been reported there on *Crotalaria verrucosa* and *Dolichos biflorus*. Much proliferated terminal brooms of *Crotalaria juncea*, of unknown cause, were brought to one of the writers from Terisi, Java, by Mr. H. de Veer in 1922. He stated that 18 per cent of a planting was affected, and diseased plants produced no seed. Schwarz[4] later published photographs of brooms on *C. juncea* and *C. anagyroides* from Buitenzorg similar to those found by Mr. de Veer but none with the extreme proliferation of some of the material from Terisi. Schwarz reports that the pods failed to ripen and the immature seed germinated in the pod.

BUREAU OF PLANT INDUSTRY
AND FOREST SERVICE,
U. S. DEPARTMENT OF AGRICULTURE.

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[4] Schwarz, M. B. Eenige ziekten van onbekenden aard bij groenbemesters. With English legends and summary. Inst. Plantenziekten. Korte Meded. 5. 1927.

A METHOD OF INOCULATION FOR PHYMATOTRICHUM ROOT ROT INVESTIGATIONS[1]

J. J. TAUBENHAUS, B. F. DANA, W. N. EZEKIEL, W. J. BACH, AND J. P. LUSK

Root rot caused by *Phymatotrichum omnivorum* (Shear) Duggar (*Ozonium omnivorum* Shear) is a very destructive disease on many hosts. Losses to cotton alone in Texas aggregate millions of dollars yearly. Though the disease has been known for more than 30 years and investigated extensively, definite progress with it has been hindered by the nearly complete failure of experimental inoculations. Thus it was not until 1923 that the first experimental proof was furnished that *Phymatotrichum omnivorum* is the cause of root rot. King[2] inoculated cotton plants in the field by inserting cotton roots, infected naturally or from artificial cultures, in a trench dug next to the roots of the plants and then filling the trench with earth. Taubenhaus and Killough[3] succeeded in inoculating cotton plants in sterilized soil with pure cultures on mulberry stems or cotton roots. Peltier, King, and Samson[4] were successful with only a single inoculation of a number that were attempted.

With the beginning of intensive study of cotton root rot at the Texas Agricultural Experiment Station, it was realized that so long as results could be based only on observational methods, studies of even such simple matters as varietal resistance could not be conducted on a strictly experimental basis. After numerous preliminary trials, the procedure outlined below was developed by the writers and tested first on cotton plants growing in deep galvanized pots in the greenhouse. It was successful there and also in plat and field tests; and was adopted as the standard procedure in the root-rot investigations at College Station and the three substations where root-rot work is in progress. Two years of further experience with the method have proved it invaluable both when used on a wholesale scale in field work, where for instance it has made definite results possible in testing cotton strains for resistance to root rot, and when used

[1] Published with the approval of the Director as contribution No. 416 of the Texas Agricultural Experiment Station.

[2] King, C. J. Cotton root rot in Arizona. Jour. Agr. Res. 23: 525–527. 1923.

[3] Taubenhaus, J. J., and D. T. Killough. Texas root rot of cotton and methods of its control. Tex. Agr. Exp. Sta. Bul. 307. 1923.

[4] Peltier, G. L., C. J. King, and R. W. Samson. Ozonium root rot. U. S. Dept. Agr., Bur. Pl. Indus., Bul. 1417. 1926.

in experimental studies on a smaller scale, such as in determining the effect of environmental conditions on the progress of root rot. (See figure 1.)

OUTLINE OF THE METHOD

Roots of plants freshly wilted from root rot are used as inoculum. Such plants invariably have advanced root lesions, since wilting from root rot does not occur until after a perceptible, and often considerable, portion of the roots has been invaded by the causal organism. Except in very dry or extremely wet ground, these root lesions are covered with a copious surface growth of the fungus. This type of inoculum is most virulent, and a higher percentage of infection is secured with it than with roots of plants that have been wilted for a longer time. Roots that are completely dead have failed to cause infection.



FIG. 1.—Cotton plants artificially inoculated with cotton root rot. Inoculum placed at left end of box nearly three months before the photograph was made.

When cotton is used, which is usually the most readily obtained inoculum, plants apparently recently wilted are pulled by hand, the tops cut off at the surface of the ground, and the roots dropped into moist sacks. Since root rot occurs in large spots and is characterized by readily recognized symptoms of wilting and the still more characteristic mycelial strands on the roots, large amounts of diseased roots can be collected with comparative ease. These roots are kept moist and used as rapidly as possible for inoculation. The inoculum, if fresh, may be successfully shipped by mail or express, or it may be kept for a few days without great loss of virulence.

Under field conditions a crowbar is used to pierce a hole in the soil 1–1½ in. from the stem of the plant to be inoculated. One or more of the fresh pieces of inoculum are placed in this hole and the soil pressed together again. The inoculum in place should lie close to the host root, and an inch

or two below the surface of the ground. In this position the infected zone on the inoculum parallels the tap-root and is usually at some point in actual contact with some of the lateral roots.

Most successful inoculations have been secured when the moisture content of the soil is high. Under non-irrigated conditions, water has been supplied locally about the plants as soon as inoculum has been inserted. A thorough soaking has given best results.

This method has proved particularly useful with crews of three men, when inoculating large plats. One man goes ahead opening holes with the crowbar, followed by a second man who inserts the inoculum, and a third who covers the holes and applies the water.

RESULTS WITH THE METHOD

Mass inoculations in the field are frequently made with one piece of inoculum inserted near the base of each cotton plant. The first results above ground appear in from ten days to two weeks, with the wilting and subsequent death of an occasional plant. In three to four weeks, 40–80 per cent of the plants are usually killed. This may be considered the result of initial, rapid infection. Following this initial killing there is almost always further infection, usually causing the death of most of the remaining plants in the next four weeks. This final loss may be indirect, as the result of spread of the disease from plants which succumbed first, or it may be merely from delayed original infection.

Thus of 840 cotton plants at College Station, Texas, inoculated early in the summer of 1928 with the roots of infected cotton plants, 346, or about 41 per cent had wilted in the next 24 days. Of 52 cotton plants inoculated with carrots that were naturally infected with root rot, 26 had succumbed to the disease in six weeks. But in both these lots, almost every plant was killed by root rot within the following three weeks, while there was still less than 1 per cent of the disease in adjoining check rows.

In making inoculations on a smaller scale for greenhouse or plat work, higher initial infections are secured by using a larger amount of inoculum for each plant and watering more liberally. For instance, cotton plants grown for soil-type studies in a series of 40 wooden containers were inoculated by this method but with four pieces of inoculum for each container. Periodically, these plants were washed out and examined. Within a week of the inoculation, abundant mycelial growth was visible along the tap-roots; after another week these portions of the tap-roots were superficially decayed; and it was only after this that many of the plants began to show any injury above ground. The inoculation proved to be successful in 39 of the 40 containers.

Although root rot is able to attack the majority of cultivated crops, yet no cross-inoculations have hitherto been reported. By the use of the inoculation method described, the writers have already made successful cross-inoculations from cotton to cowpeas, tomatoes, alfalfa, guar, lima beans, eggplant, peach and prune trees. Likewise, cotton, sugar beets, cowpeas, soy-beans, sunflowers, pear and apple trees, and grape vines have been successfully inoculated with carrot inoculum. Sweet potatoes and beets have been used to infect cotton; and the roots of a diseased jujube tree have infected a mulberry tree and cotton.

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PHYTOPATHOLOGICAL NOTES

A New Cotton Wilt[1]—Early in the fall of 1927, our attention was called to a disease which was said to cause heavy damage to cotton in Ellis County, Texas. Some plants were examined and found to be affected by a wilt which appeared different from the common cotton wilt caused by *Fusarium vasinfectum* Atk. During 1928, the disease has been found prevalent in Ellis County, in heavy clay soils and in fields in which root rot [*Phymatotrichum omnivorum* (Shear) Duggar] was also prevalent. We have recently found what appears to be the same disease in El Paso County and Brazos County. These three counties are in widely separated sections of the state, so that a wide distribution at least in Texas is indicated.

Superficial symptoms of the disease resemble those of the common Fusarium wilt. The plants are decidedly stunted, especially if infection occurs early in the season; and are peculiarly branched with abnormally short, stout joints. With the advance of the disease the plant sheds its leaves and the branches become dull in color. In some cases, plants die from the tops, and new growth appears on the lower part of the main stem. Only a small percentage of the affected plants succeed in making a boll or two of cotton. Occasionally, infected plants are so dwarfed and stunted as to take on the appearance of a rosette.

The new disease may be distinguished from Fusarium wilt by splitting the stems. In both diseases there is blackening; but in the case of the new wilt the discolored area is confined mostly to the interior cylinder of both roots and stems, while with typical Fusarium wilt it is mostly in the outer, woody tissue. (See Fig. 1.) In the new disease, the discoloration is more pronounced in the lower part of the plant and is progressively less higher on the stem.

About 400 isolations have been made from roots and stems of affected plants from a field in Ellis County in which there was 60 per cent loss from the disease, and parallel cultures made from typical Fusarium wilt of cotton from Athens in east Texas, where this disease is so prevalent. A pure culture of *Fusarium vasinfectum* was obtained in practically every case from either roots or stems of the cotton from Athens. On the other hand, many organisms were isolated from the Ellis County plants. One of these is a *Fusarium* which has from the beginning appeared distinct from *F. vasinfectum*. The other organisms isolated are species of *Alternaria, Sclerotinia, Phoma, Phomopsis,* and *Helminthosporium*. Work is now in progress to determine the pathogenicity of any or all of these organisms.

[1] Published with the approval of the Director as contribution No. 417 of the Texas Agricultural Experiment Station.

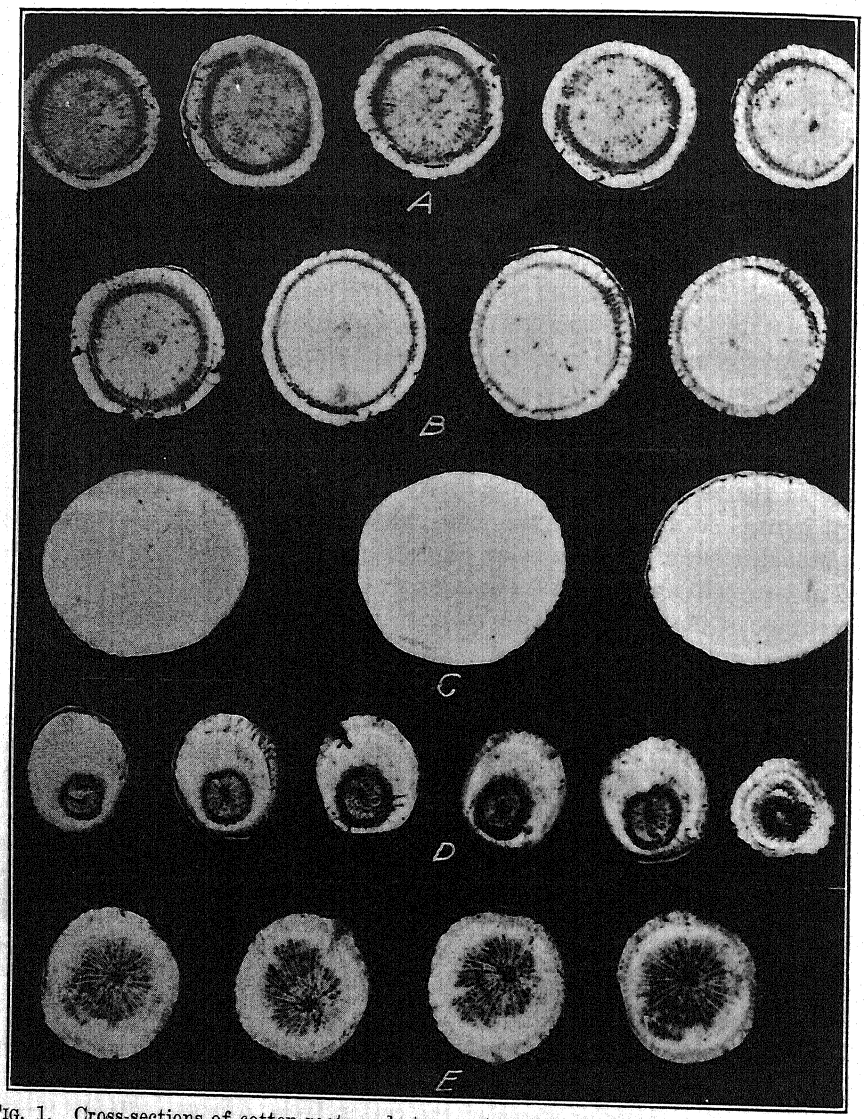


FIG. 1. Cross-sections of cotton roots and stems. A and B, the wilt caused by *Fusarium vasinfectum*. C, sections of normal plants for comparison. D and E, the new cotton wilt.

It is of particular significance that this disease has been found in the same soils and in the same fields as those in which root rot is destructive. Workers with the old Fusarium wilt of cotton in the United States have long considered this disease as peculiar to light, sandy soils. Some of us[2] have

[2] Taubenhaus, J. J., Walter N. Ezekiel, and D. J. Killough. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity of the soil. Texas Agr. Exp. Sta. Bul. 389. 1928.

recently shown that this relation is apparently a matter of soil acidity. Fusarium wilt appears to be confined mostly to soils more acid than pH 6.5 —7.0. On the other hand, Phymatotrichum root rot seems to be destructive only on soils that are more alkaline than this. The two diseases are, in fact, never found in destructive abundance in the same parts of the same field; and the Fusarium wilt has not been of considerable importance in the regions in which root rot has been abundant. In this new disease we are thus confronted for the first time with a destructive cotton wilt in the heavy black lands.

Search through the American literature failed to reval any description of the disease in question. Fahmy,[3] however, makes brief mention of an apparently similar cotton disease which he found to occur in Upper Egypt. He describes a peculiar discoloration in resistant varieties such as the Ashmouni or Zagora varieties which was very different from the common Fusarium wilt, and refers to this as "Upper Egypt" discoloration for the reason that it was observed in Upper Egypt where the Ashmouni and Zagora varieties are grown. This discoloration was localized in a part of the central cylinder of the root, hypocotyl, or lower part of the stem. Isolations made by Fahmy from these discolored portions yielded a *Fusarium* which failed to infect the Ashmouni or Zagora varieties and which he accordingly considered non-parasitic. It is regrettable that Fahmy's figure of the "Upper Egypt" discoloration is not very distinct. From his description, however, the Texas disease is much the same as that found in Upper Egypt. Should this prove true we could not agree with Fahmy that the discoloration in the roots is of no consequence, at least not under our conditions. In Texas, this interior discoloration of the central cylinder of the roots and stems of cotton is associated with a definite and serious wilt disease of the plants.—J. J. TAUBENHAUS, W. N. EZEKIEL, and H. E. REA, Texas Agricultural Experiment Station, College Station, Texas.

Dusting seed for oat smuts.—Until the recent work was done in Ohio with formaldehyde and iodine dispersed in infusorial earth,[1] no satisfactory dusts had been developed for controlling the two smuts of oats in spite of the fact that extension men and growers have been asking for a dust treatment comparable to the one for stinking smut of wheat. An opportunity to compare these Ohio dusts with some organic mercury dusts under New York conditions presented itself in the spring of 1928.

[3] Fahmy, Tewfik. The Fusarium disease of cotton (wilt) and its control. Min. Agr. Egypt, Tech. and Sci. Service Bul. 74. 1928.

[1] Sayre, J. D., and R. C. Thomas. Formaldehyde and iodine dusts for the control of oat smut. Ohio Agr. Exp. Sta. Bimonthly Bul. 13: 19–21. 1928.

The results of this experiment were so striking and apparently so significant that a short note regarding them seems justified. Of course no sweeping conclusions can be drawn from a single experiment, but the results of this one are nevertheless of interest. A supply of glumed oat seed naturally infected was obtained through the courtesy of Dr. W. H. Tisdale of the E. I. Du Pont de Nemours and Company. Thirteen lots of seed placed in separate mason jars were well shaken with the different dusts at the rate of 3 ounces to the bushel. A fourteenth lot was left untreated as a check.

The chemicals used in the experiment are as follows: KlC, KlB and 68, all organic mercury compounds from E. I. Du Pont de Nemours and Company, Wilmington, Delaware; 200, 197, 196, 195, 194, 193, 192, and 189, also organic mercury compounds, from the Agricultural Department of the Bayer Company, Inc., New York City.[2] Iodine, 1 per cent in infusorial earth, and Smuttox which is 4 per cent formaldehyde in infusorial earth were obtained from Grasselli Chemical Company, Cleveland, Ohio.

TABLE 1.—*Mean percentage of oat smut in six replicated rod rows for each dust treatment*

No.	Treatment	Smut in per cent		
		Total	Loose smut	Covered smut
1	Check	[c]12.62 ± 0.06	[a]3.33	[a]10.2
2	DuPont KlC	0.11 ± 0.01	0.00	0.11
3	DuPont KlB	0.33 ± 0.009	0.11	0.22
4	DuPont 68	3.22 ± 0.06	[a]0.93	[a]2.20
5	Grasselli Iodine	13.11 ± 0.69	[b]0.40	[b]9.92
6	Grasselli Smuttox	0.33 ± 0.01	[a]0.00	[a]0.27
7	Bayer 200	1.67 ± 0.03	[a]0.13	[a]1.40
8	Bayer 197	4.33 ± 0.04	0.78	3.56
9	Bayer 196	2.67 ± 0.04	0.61	2.06
10	Bayer 195	3.44 ± 0.03	[a]1.00	[a]2.47
11	Bayer 194	3.44 ± 0.04	0.50	2.94
12	Bayer 193	2.06 ± 0.03	0.28	1.78
13	Bayer 192	3.17 ± 0.04	[a]0.47	[a]2.81
14	Bayer 189	7.67 ± 0.07	[a]1.53	[a]6.00

[a] Only 1500 panicles in the series.

[b] Only 1200 panicles in the series.

[c] Discrepancies between total smut and the sum of the individual smut species occur because the two smuts were not counted separately in one or more rows.

[2] The fungicide, insecticide, and disinfectant departments of these two companies have consolidated under a new subsidiary, the Bayer-Semesan Company, Inc., since these materials were procured.

Seed of each treatment were hand planted in rod rows replicated 6 times in soil which was well prepared. The hands were carefully cleaned between each sample. After the oats had headed, 100 contiguous panicles were counted in 3 different places in each rod row making a total of 1800 panicles for each treatment and 2000 panicles for the check, because 200 additional heads were counted in the last check row of the series. Except in one or two cases the two smuts were counted separately. The experimental results are set forth in table 1.

The facts brought out by the data are: (a) that Du Pont dusts KlC and KlB[3] and Grasselli Smuttox controlled both smuts very satisfactorily; (b) that Bayer dust 200 is slightly less effective, while Bayer nos. 197, 196, 195, 194, 193, and 192 are intermediate, and Bayer 189 is ineffective; (c) that Du Pont 68 is in a class with most of the Bayer dusts in this experiment; (d) that the iodine dust was inefficient under the conditions of the Ithaca experiments.—JAMES G. HORSFALL, Cornell University, Ithaca, New York.

A new and striking abnormality in potato plants.—An abnormal condition in potatoes, believed to have been undescribed hitherto, was observed during the summer of 1928 in a single crop of Rural Russet potatoes on Vancouver Island, British Columbia, Canada.

The most striking features in all plants obviously affected was the presence, below the surface of the ground, of a tumor-like tuber on every stem. The stem, arising from the set, entered this swelling at its base and, emerging from the apex, continued on to the light and air to bear leaves. Although the tumor bears eyes, it is not a normal tuber simply penetrated by the stem, but is an unusual growth of the stem tissues.

Other symptoms apparently associated with this peculiar development are a dwarfed plant and fewer and smaller tubers, most of which are malformed. No symptoms such as mottling or wrinkling of the leaves were noted, but the dwarfed and immature condition of the aerial part of the plant was sufficiently definite to enable one to select severely affected plants in the field with ease. The vines in such cases would be about 3 inches in height and quite green, as compared with those of normal plants approximately 16 inches high and approaching maturity. Between these two extremes there was a gradation, and here the separation of normal from abnormal plants was more difficult without examining the stem below the ground for the characteristic growth. In all cases, however, plants exhibiting the tumor of the stem were noticeably dwarfed.

[3] Since these experiments were performed, the Bayer-Semesan Company has placed on the market a dust intermediate between KlC and KlB, under the trade name, "Ceresan."

Approximately 1 per cent of the plants in the crop showed this abnormality, and the grower states that the same condition has been present to a greater extent in past years.

As the problem is being investigated with a view to ascertaining whether it is a further addition to the list of virus diseases affecting the potato, any related information or material would be greatly appreciated by the author.—L. M. BLACK, Department of Botany, The University of British Columbia, Vancouver, B. C., Canada.

Cultures of sclerotial fungi.—During the past 15 years I have been accumulating cultures of sclerotial fungi of all kinds, in connection with studies on the taxonomy of *Sclerotinia* and *Botrytis*. The total number of strains which we have had in culture to date approximate 1600. While some of these have died, we still have growing in stock approximately 1000 strains. Under a special grant from the Heckscher Foundation for Research in Cornell University, we are able to maintain these cultures year after year. Many workers throughout the United States and in other parts of the world have, from time to time, made many contributions of specimens and cultures of sclerotial fungi for our use.

We should greatly appreciate it if all workers who may have occasion to collect or isolate sclerotial fungi of any kind would send us cultures or freshly collected specimens, preferably both, whenever they come to hand. We are glad to make any of our cultures available to other workers in so far as is consistent with our obligations to contributors and the progress of our own investigations, believing that in this way we will be rendering service to our colleagues at very little inconvenience to ourselves.

We are particularly anxious to get cultures of any newly described species whether they be of *Botrytis, Sclerotinia* or other sclerotial fungi. Workers on any of these forms need have no hesitancy in sending such cultures or forms on which they may be working, as we shall be very glad to maintain them in culture under such restrictions as to distribution or use by us in our investigations as the sender desires to make. Full data as to time, host, place of collection, etc., should accompany the sendings.

Hearty coöperation is invited of all workers or collectors of these forms in this effort to maintain a large and representative culture collection of sclerotial fungi.—H. H. WHETZEL, Cornell University, Ithaca, New York.

BOOK REVIEW

Principles of Plant Pathology. By Charles Elmer Owens. Edition I, 929 pages, 222 figures. 1928. John Wiley and Sons, Inc.

Although a number of excellent treatises on different phases of plant pathology have been published in recent years, their nature has been such as to require the student to purchase several books in order to gain a comprehensive view of the subject. It is gratifying, therefore, to receive the new book by Owens, entitled "Principles of Plant Pathology." As the title indicates, the author presents the principles of the science in a way that appears to be especially usable in teaching undergraduate students.

Part I of the book consists of a discussion of topics of general interest concerning the nature, cause, and control of plant diseases, comprising an interesting review of the history of plant pathology and its place relative to other branches of science. This part is unique in furnishing the student with material on which to base a broad view of plant pathology.

Chapters are devoted to such subjects as symptoms of plant diseases, and their classification, methods of investigating plant diseases, and the effect of external factors on plant diseases. Control measures are discussed particularly in chapters on fungicides, disease resistance, and quarantine. The relation of insects to plant disease and problems of storage, transportation, and marketing are also treated.

Part II presents specific diseases selected from a wide field representing types of causal agents and types of symptoms. The history and distribution of the plants attacked, and symptoms and economic importance of diseases are discussed. This discussion is followed by a description of the organisms, their life cycles, and control measures. At the end of each chapter a valuable supplementary list of diseases is given together with selected references, also directions for laboratory work, and a list of review questions. This sort of discussion is helpful both to the student and to the instructor.

While the general scheme of Part II is based on a mycological classification of the causal agents, this is subservient to the presentation of types and basic principles. The bibliographies following the chapters are well selected and the book is profusely illustrated with excellent photographs and line drawings.

The subject matter on plant pathology has become so voluminous that in the average course it is hopeless to try to present more than the leading and essential facts. Dr. Owens' book serves this purpose well since its outstanding feature is its general treatment of the subject, bringing to the student the important points of plant pathology. This is well summed up in the title "Principles of Plant Pathology." I believe the use of this book will prove it a worthy text.

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William H. Wright

PHYTOPATHOLOGY

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AN ACTINOMYCETE THE CAUSE OF SOIL ROT OR POX IN SWEET POTATOES

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During the past six years investigations have been conducted on the cause of pox or soil rot of sweet potatoes. The results of these investigations are such as to justify a preliminary account pending more complete report on the problem, study of which is being continued.

Studies of the pox disease of sweet potatoes were undertaken in 1920 in order to confirm the results of Elliott (1), who first reported that the disease was caused by a slime mold, *Cystospora batata* Ell. Elliott's results were later confirmed by Taubenhaus (13). The author's initial studies in duplicating the work of Elliott and Taubenhaus gave negative results in recovering or locating a slime mold from typical pox lesions on sweet potatoes. Mature pox lesions for cytological study were differentiated with Flemming's triple stain. Studies of these mature lesions on fleshy roots failed to reveal the presence of any foreign organism resembling a slime mold. It was reported (4) that "Products of metabolism and the results of cellular disturbances of unknown cause were consistently observed and in part assumed the morphological character of the so-called cysts, as described by Elliott. These cyst-like bodies possessed no differentiated contents, and were interpreted as probably the products of metabolism in the form of reserve substances." Further work by the writer (5), reported in 1922, failed to confirm the results reported by Elliott (1) and Taubenhaus (13) that pox of sweet potatoes was caused by *Cystospora batata* Ell.

Manns (6) reports that "In 1914 we suspected the cause of pox may be due to an Actinomycete found associated with the disease in our cultures, but results were not consistent." Taubenhaus (13), then an assistant in the department, reported upon these findings in 1918. Manns (6) found by unmounting pox material which had been stained by Elliott with Flemming's triple stain, and restaining with Ziehl's carbol-fuchsin, that in every case there was evident an Actinomycete, which may have been the cause of the disease. An experiment was established by growing sprouts in Mason jars to learn the influence of lime and manure on increasing the disease. Ground lesions of mature pox and scrapings from culture plates were used

by Manns for inoculation of sprouts in two jars, and he reports in the case of ground-lesion inoculation that "small roots were eaten off with small black lesions and one large root was rotted off." In the case of scrapings from culture plates for inoculation, it is reported that "several pox pits showed on the main roots and many rootlets were injured." This experiment was run for over six months and no duplication of work on reisolation is reported. No controls were used with soil and manure to which were added the equivalent amount of straight agar. The introduction of agar alone into such a mixture of soil and manure would naturally force into growth any fungi and bacterial flora present and produce conditions inhibitive to normal root development.

In writing of the work conducted during 1923, it was stated (6) that bacteriological staining methods were employed and found to be superior to Flemming's triple stain for differentiating organisms in the necrotic tissue of pox lesions. The safranin in the triple stain possesses only slight affinity for a fungus which to all appearances resembles an Actinomycete in its morphological characters. Ziehl's carbol-fuchsin stain was found very efficient, but for careful differentiating it was necessary to destain. A stain for bacterial flagella (11) was found very efficient. A fungus that is easily stained by the methods described above was found consistently in the lesions on fleshy roots. The necrotic tissue was found invaded with an abundance of the mycelium. The mycelium was not widely distributed but appeared in the necrotic tissue in definite pockets.

In 1925, Manns (7) reports as follows: "By improved cultural technique we have been able to take this Actinomycete from pox lesions in pure culture by growing the crushed lesion in straight agar medium, 1.2 per cent, in the absence of all nutrients. Such a medium inhibits most organisms, and *Actinomyces poolensis* pushes out almost pure. We have been able to prove its activity in producing 'soil sickness' by placing the contents of a dozen culture dishes in a quart mason fruit jar using sterile sand and proper sweet potato fertilizer." The two inoculated sprouts used for illustration show a complete collapse of the zone of root growth as well as retarded growth but no pox lesions such as are found under natural conditions of root infection in the field.

Further cytological studies (8) were continued with the pox lesions during 1924. Material showing lesions only on the rootlets was used, the object being further to confirm previous work and the results of studies with mature lesions on fleshy roots. Consistent evidence was found of the presence in lesions on rootlets of apparently the same pathogene as was found in the lesions on fleshy roots. The characters of this fungus from a cultural and morphological study are identical with those of the genus

Actinomyces. In addition to the stains previously used, it was found that Bismarck brown is very satisfactory for differentiating the pathogene. The fungus was always found associated with tissue, principally in the cortex of young roots that showed complete collapse. There was little evidence of any progressive development of the pathogene, as the lesions were very restricted on the rootlets, as in the case of infection on fleshy roots.

Some direct inoculation experiments were carried out with two strains of *Actinomyces* sp. isolated by Dr. Manns. Tall pyrex beakers of 1-liter capacity were used for this work with a layer of moist absorbent cotton for a medium, and the beakers were plugged with cotton. Sprouts grown from disinfected sweet potatoes of the Big Leaf Up River and Vineless Jersey varieties were used. Clean sprouts were secured, thoroughly washed in distilled water, and placed in the sterile beakers. There was no difficulty in maintaining the sprouts for a period of four weeks and more. Four series of four beakers each were protected from the light by being wrapped in black paper. The inoculum used in this experiment was from colonies on agar plates and was placed directly on the primary and secondary roots. Room temperature, approximately 68° F., and a temperature of about 85° F. were used. In only one instance did a lesion develop that resembled a pox lesion. The result of this experiment was considered negative.

In 1926 further work was reported (9) upon as follows: Eight roots showing abundant lesions were selected, carefully washed in distilled water, placed in sterile beakers, and submerged in distilled water. The water was changed twice a week. For a period of about four weeks thereafter the roots showed no evidence of breakdown. The experiment was started October 24, 1925, and this series of roots removed for culture isolations November 20. A series of four lesions was removed from each root and cultured on plain agar, which had proved most satisfactory in previous studies for the pathogene under manipulation. With this series of 40 plate cultures, 8 additional plate cultures were made with lesions from untreated roots as a control. It was found at the end of eight days that the plates from lesions of the potatoes which had been submerged showed on the average 50–75 per cent more colonies of the Actinomycete than the control plates. This proved that the incubation of the pox roots in water was a factor in activating the fungus. However, in no instance was there evidence that the organism had progressed so as to extend the original limits of development of the lesions. This particular character may be associated with the resistance of the root or the original protective zone of tissue cutting out the lesion. According to our observations, inception of the disease under field conditions occurs with young, actively growing secondary or fleshy roots and tissue, or at the eye points or rootlets from primary fleshy roots. This experiment was then followed with some direct inoculations.

Eight disease-free fleshy roots were disinfected, carefully washed, and placed in sterile beakers with moist cotton for forcing of roots, on October 24, 1925. Roots were well established in four weeks. Lesions from poxed roots that had been incubated in water cultures as previously described were then placed at the point of origin of the rootlets. In 10 days diseased areas were observed at points of contact of the inoculum which looked like typically mature pox lesions. The resulting diseased areas or lesions with one exception were light to dark brown in color and not more than $\frac{1}{8}$ in. in diameter and non-progressive. With one exception in the 18 inoculations, the resulting lesions were typical in every respect of small, developing pox lesions. The lesions resulting from these inoculations as well as the inoculum were plated out on plain agar on December 1. In seven days the typical Actinomycete under observation appeared in all plates. A series of plates was also made from dried pox lesions for control. The colonies were more abundant in the plates made from the newly developed lesions and from the inoculum than in the plates made from dried material. This further confirms the evidence previously reported that incubation of the lesions activated the pathogene.

In another experiment a disease-free root was disinfected and planted in sterile soil. This was kept moist with distilled water and forced until emerging root points were first showing. The soil was removed, and on the upper surface at nine different emerging root points, direct inoculations were made with pox lesions that had been soaked for 13 days in distilled water. Inoculations were made December 20, the inoculation court marked with a circle of India ink, and then the soil was carefully replaced so as not to disturb the pieces of inoculum. The resulting lesions were examined on January 11, 1926, and removed for plating cultures. In all except one instance the lesions were restricted, pitlike, and non-progressive. The results were positive with respect to production of diseased lesions. The one exception in this inoculation experiment was a lesion in which the soft-rot fungus was associated with the Actinomycete so that the resulting lesion was progressive as compared with the remaining eight which appeared as young pox lesions. The removed lesions were divided into three pieces for crushing and plating out in plain agar and nutrient glucose agar. The same specific Actinomycete under observation appeared in the plate cultures, but for some unexplained reason the colonies were not so abundant as those resulting from previous inoculations. It is possible that the pathogene had not sporulated or developed to the same extent as in the previous inoculation work.

The results of these direct inoculations indicated the presence of an organism that was pathogenic and capable of producing lesions typical of the pox disease. During the fall of 1926, fresh specimens of the pox disease

on fleshy roots of sweet potatoes were collected for more detailed isolation studies. Plain agar was used for the isolation work, as this medium eliminates much of the contamination including bacteria and other fungi naturally following the initial collapse of the host tissue. The diseased fleshy roots were thoroughly washed in distilled water and then kept in a moist chamber for nine days at a temperature of 33° C. Typical pox lesions were removed and crushed under sterile conditions and plated out in plain agar. The colonies of the *Actinomyces* sp. showing growth on these agar plates were much more numerous than colonies from finely crushed lesions that were not kept in the moist chamber. This procedure again appears to have activated the *Actinomyces* in the lesions. The isolation work failed to show a consistent appearance of any single type of *Actinomyces*. It was necessary to incubate the culture plates at a temperature of 33° C. for maximum growth of the colonies. The colonies or growth from diseased tissue was evident in the poured plates within five to eight days. Three types of *Actinomyces* were isolated and carried as pure cultures for further inoculation work. These three types varied perceptibly in growth and color characters on Ashby's, cornmeal, synthetic, Endo, and nutrient agar. Inoculations were first started on fresh slices of fleshy sweet potato roots in sterile agar plates. One of the three types was found to be capable of producing a restricted lesion, indicating that it was pathogenic. This particular type was named for convenience, *Actinomyces p.*, and will be referred to as such in the discussion as the *Actinomyces* causing pox of sweet potatoes.

A cultural difference was established for *Actinomyces p.* compared with *A. poolensis* and two strains of *A. scabies*. The following media were found most satisfactory for differentiating the fungi: Ashby's, synthetic, Endo, and nutrient agar. Ashby's medium proved to be most satisfactory for rapid vegetation and sporulating growth. The culture of *A. poolensis* and one of *A. scabies* were received from Waksman, while the other culture of *A. scabies* was isolated by Manns from scab lesions on white potatoes.

In addition to extensive cultural studies on differentiation, some work was undertaken to determine the response of these cultures to media to which pH indicators were added. Synthetic and Ashby's media were used, and to each 10 cc. of media there was added 1 cc. of indicator. The procedure followed was to inoculate the tube of melted agar and then add the indicator and pour into sterile culture plates. The first indicator used was brom cresol purple with a pH range of 5.2 to 6.8. Two uninoculated plates or controls of Ashby's medium were used for comparison and the plates were read after four days at a temperature of 33° C. With this indicator *Actinomyces p.* and *A. scabies* produced a change to yellow, indicating the acid reaction, while *A. poolensis* increased the intensity of the purple

color, showing the alkaline reaction. Thymol blue with a pH range of 1.2–2.8 and 8–9 was used with synthetic agar. In this series the plates were checked against indicators for final readings. *Actinomyces p.* and *A. scabies* showed 2.4 readings while *A. poolensis* and controls showed 2.8 readings. Brom cresol purple with a pH range of 5.2 to 6.8 with cultures on a synthetic medium showed that the controls and *A. poolensis* gave readings of 6.4 while *Actinomyces p.* and *A. scabies* gave readings of 5.4. These studies further supported the specific difference already established culturally between *A. poolensis, Actinomyces p.*, and *A. scabies.*

Temperature requirements are very important in relation to growth in culture as well as for infection studies of *Actinomyces p.* Room temperature is not favorable for optimum growth or infection. The optimum temperature range for this fungus was found to be between 30° and 37° C. At 52° C. very slight growth was made.

The first series of inoculation tests was conducted on slices of fleshy sweet-potato roots. Disease-free roots were selected and disinfected. Slices averaging ¼ in. in thickness were made and placed immediately on poured plates of plain agar. These plates were incubated for two days before being used for inoculation. The inoculum consisted of a single colony (¼–½ in. in diameter) transfer of *Actinomyces p.* from plates 10–14 days old. The series of tests were conducted under three ranges of temperatures, *viz.*, 20° C., 33° C., and 37° C. *Actinomyces p.* proved pathogenic under these conditions in developing a lesion restricted to the size of the inoculum within 48 hours at a temperature of 33° C., as illustrated in Plate III, A. Only a slight trace of infection occurred at room temperature. The lesion was typically black in appearance, and at the end of six days was conspicuously sunken and slightly larger than the inoculum. At 37° C. a slight, effused white growth of the fungus occurred, indicating sporulation. This method of inoculation was extended to include white potato, dahlia, carrot, beet, and turnip roots. Positive results were secured except in the case of carrot and dahlia. On white potato the character of the lesion was similar in appearance to that on sweet potato, whereas with beet and turnip the lesion was brown instead of black. The developing lesions were cut out and divided in half: one piece was used for reisolation work and the other killed and prepared for sectioning. This double check later proved that there were no contaminations.

The same method was used in further inoculations of sweet potato and white potato, but using in addition to *Actinomyces p.* the two strains of *A. scabies* and *A. poolensis.* The results secured from inoculations with *A. poolensis* and *A. scabies* received from Waksman were negative on both hosts, while *Actinomyces p.* and the strain of *A. scabies* isolated by Manns produced infection. This strain of *A. scabies* showed more evidence of being pathogenic on sweet potato than on white potato.

The growth of sprouts and root development were secured under sterile conditions by including the "eyes" of white potatoes and the growing points of sweet potatoes in the slices for use on poured plates of plain agar. Plates made up in this manner were kept at room temperature until roots or sprouts had grown enough to be inoculated. In this manner inoculations were made possible on such growing parts of the hosts as are not easily inoculated when the plants are grown in soil.

As soon as root points emerged from the fleshy roots of sweet potato they were inoculated with *Actinomyces p.*, and the resulting lesions were typical of those found under natural conditions. These results confirm field observations which always indicate that typical pox lesions on fleshy roots have their inception through rootlet infection. Growing rootlets an inch or more in length were next inoculated at the point of emergence from fleshy roots, which resulted in the development of typical pox lesions, as illustrated in Plate III, G. The resulting lesions secured by this method of inoculation on rootlets were restricted in growth to approximately the size of the inoculum. No extensive or progressive type of lesion occurred as a result of these inoculations. Inoculations on the uninjured surface or epidermis of fleshy sweet potato roots always gave negative results.

The next series of inoculations with *Actinomyces p.* was made at different points on rootlets which developed from slices of sweet potatoes planted on agar plates. The inoculum varied from a whole to half a colony and was placed directly on the rootlets growing on the surface of the plain agar plates. The resulting lesions were typically black in appearance and restricted in size to that of the inoculum. This typifies lesions on rootlets found under field conditions. Such rootlet infection under field conditions accounts for the sloughing of the root system and the general retardation of growth above ground. These rootlet lesions, when their pathological histology is studied, show much greater collapse of the tissue than is found in lesions on fleshy roots.

Inoculation of sprouts was next undertaken to parallel infections found under natural field conditions. Sweet potatoes were disinfected and bedded in sterile sand for propagation of sprouts. When sprouts reached 6–10 inches in growth, they were pulled. These sprouts were then placed in sterile cylindrical beakers of 1,000-cc. capacity with a layer of moistened cotton in the bottom and closed at the top with a plug of absorbent cotton. Most of the foliage growth protruded beyond the mouth of the beaker and cotton plug. All beakers were wrapped in black paper in order that light would not inhibit root development. Sprouts were carried under these conditions for three days before being inoculated. Development of sprout and root under these conditions occurred as normally as under bedded condi-

tions. In seven days, extensive root development of the controls occurred at a temperature of 33° C. and the layer of moistened cotton was ramified by the growing rootlets. Cultures of *Actinomyces p.* were prepared in 250-cc. flasks which contained 40 cc. of nutrient agar. When cultures were 14 days old, sterile glass beads and 125 cc. of sterile water were added. The flasks were shaken and the medium thoroughly broken up as in the form of a thin gel. The sprouts to be inoculated were then removed from the beakers and the entire zone of root development and part of the stem were immersed in this inoculum. These sprouts were replaced immediately in the sterile beakers and kept at a temperature of 38° C. The first series of sprouts was immersed for one minute, but it was later found that a three-minute immersion was more conducive to maximum development of lesions. This type of inoculation proved very successful in producing lesions similar to those found on sweet-potato sprouts under natural field conditions. Typical lesions appeared on various growing parts of the sprout within 7–10 days, the incubation period being longer than that following the previous types of inoculation. The origin of lesions appearing on the primary root of the sprouts within the zone of rootlet development always was a rootlet infection, as illustrated in Plate III, E and F. Typical, black, restricted lesions occurred at various points on the rootlets, as illustrated in Plate III, H. Lesions were removed for reisolation work and for study of their pathological histology. Consistent proof was secured that this phase of inoculation work was being conducted with a pure strain of fungus that was identical with the original *Actinomyces p.* Although the cultures used in these experiments were sporulated, it was not determined whether infection resulted from the spore or from the vegetative growth of the fungus.

A type of lesion occurred as a result of this method of inoculation that has not been previously described for sweet potatoes or observed under natural conditions. In instances in which the sprout was immersed in the inoculum above the zone of root development so as to include the green stem part, lesions developed which were different in appearance from those found on the root end of the sprout. These lesions first appeared as black pin points of infection, and, gradually enlarging more lengthwise than crosswise of the stem, grew to as much as 10 mm. in length and 4 mm. in width. Since this infection occurred above the zone of root growth, the infection can only be associated with entrance through the stomata. At maturity these lesions have a blackened margin and the center is brown and sunken. They are very slow in developing and do not appear until 4–7 days after lesions first appear on the roots. These lesions were cultured and prepared for cytological studies. The *Actinomyces p.* used in the inoculations was also recovered in pure condition.

Another series of experiments was established by inoculating sprouts as above described with *A. poolensis* and *A. scabies,* the latter fungus being from the culture isolated by Manns. Results were negative with both fungi. The failure of *A. scabies* to produce infection under these conditions may be attributed to improper technique or to the fact that it is weakly pathogenic on sweet potato sprouts. Sprouts inoculated under these conditions with *Actinomyces p.* and *A. scabies* always showed a retarded growth and root development compared with controls, while inoculation with *A. poolensis* had no such effect. This reaction tends to indicate that a toxin or some product of the fungous metabolism retarded growth. A preliminary test was made under controlled conditions of the effect of the filtrate from cultures of *A. scabies* on root growth of white potatoes, and the results of tissue collapse were very positive. Further detailed work should be conducted, however, before the preliminary results are reported.

The histological preparations of the diseased lesions on sweet potatoes were found to be most easily differentiated with the iron alum haemotoxylin stain. The fungus observed in all lesions prepared for study resembled that used in all inoculations and studied in pure culture. The development and effect of the fungus in the host tissue are identical in both the natural and the artificially produced lesions. The fungus appears to be inter- as well as intra-cellular. It is most conspicuous within the host cells and usually is evident as a mass in the area of the necrotic lesion. The cells making up the lesion usually retain their identity but are devoid of their cellular contents. The parasitism is not associated with a complete collapse of all the cellular tissue making up the lesion, as is indicated by the macroscopic appearance of the lesion.

DISCUSSION

Halstead (2) was apparently the first to associate a fungus with sweet-potato pox, naming the causal organism *Acrocystis batatas* E. and Hals. Elliott (1) interprets the illustrations of Halstead as apparently various stages in the life history of the plasmodium *Cystospora batata,* which he described as the cause of pox. Taubenhaus (13) states that "the genus *Acrocystis,* as originally described by Halstead, is not valid and is non-existent. The fungus *Acrocystis batatas* was mistaken by Halstead for a myxomycete which Elliott has named *Cystospora batata,* the true cause of pox (soil rot)." After making extensive studies of various prepared materials of diseased roots and fleshy roots of sweet potatoes, the writer realizes how easily various foreign bodies were mistaken by these early investigators for stages in the life history of pathogenes. Species of *Rhizoctonia, Pythium,* and undetermined Phycomycetes are often found associated with different disease symptoms on sweet potato, in addition to the fungous diseases known as black rot and soil stain. Nematodes are not uncommon in some of the mature pox lesions and are also found in the rootlets. Nema-

todes in various stages of their life history are found within the tissue of rootlets and fleshy roots as contamination of lesions. Histological preparations of the pox lesion with infestation of nematodes naturally show various types of sections through young and mature nematodes as well as egg masses. In many instances such sections are not unlike certain illustrations and descriptions given for *Acrocystis batatas* and *Cystospora batata*. Poole and Schmidt (12) have given careful study to the nematode disease of sweet potatoes. It is stated that "on susceptible varieties the symptoms were so-called knots and scabs on stems, roots and potatoes. On the susceptible varieties malformation was produced; scabby areas, followed by pit-like rots, were prominent; but raised pustule-like areas were not very abundant on potatoes." Their illustrations of scab and pit areas on sprouting points are not unlike symptoms of pox. In the case of small potatoes and roots in which female nematodes were abundant, the effect is not unlike the initial symptoms of pox lesions. Their studies further indicate the possibilities of confusion in studying the pox disease, which may account in part for some of the descriptions reported by the earlier investigators.

The fungus *Actinomyces poolensis* was used for comparison, because Taubenhaus (13) has reported this fungus as a "superficial wound parasite, usually found following the pox spots produced by *Cystospora batata*. . . . Inoculation experiments with pure cultures of this organism showed that it is capable of producing a spot which, although not resembling pox, may penetrate equally deep in the host tissue."

The results of inoculations showed that *Actinomyces p.* and *A. scabies* were pathogenic on cut slices of sweet potato and white potato as well as on rootlets from sprouted pieces of their hosts developed under sterile conditions. When inoculation of sweet potato sprouts was undertaken with *A. scabies* isolated by Manns from white potato by immersion in an inoculum of macerated colonies, the results were negative on the roots as well as on the stem. It is possible the period of immersion in the inoculum was too short. This method should be as effective as direct inoculation with colonies of the fungus. The infection of the roots and stems of sweet potato sprouts with *Actinomyces p.* is similar to the infection of stems and stolons of white potatoes by *A. scabies* as reported by Jones *et al.* (3). This type of infection on these two hosts is associated with an initial crippling of the root system, which is indicated by reduced yields where heavy infection occurred. However, root infection of white potatoes by *A. scabies* has not been demonstrated by investigators in this country as an important phase of the disease. Millard and Burr (10) demonstrated root infection of white potato with *A. scabies* in Great Britain. They report that scab rarely appears to exert any adverse effect on yield but consider that reduction of yields in America may be accounted for by root infection.

Actinomyces p. and *A. scabies* are easily differentiated from a cultural standpoint, and were shown by cross inoculations to be pathogenic for sweet potato and white potato, respectively. Millard and Burr (10) have established 11 species of *Actinomyces* that were found to produce scab on white potatoes. Their work, as well as that of Wollenweber (14), further shows the possible confusion that may exist in working with this group of *Actinomyces*. Because of this fact, it would seem most feasible to determine further possible relations between other species already described and this Actinomycete on sweet potatoes.

It is significant that there is a parallel response between the scab of white potatoes and the pox of sweet potatoes to sulphur as a fungicide. The effect of acid soil conditions is an inhibiting factor in the prevalence of these two diseases. It has been proved in field experiments that the amount of soil acidity tolerated is much greater for the sweet potato than for the white potato.

SUMMARY

Studies of the pox or soil rot in sweet potatoes have failed to produce evidence that it is caused by either the fungus *Acrocystis batatas* E. and Hals. or the slime mold *Cystospora batata* Elliott.

A species of *Actinomyces*, for convenience referred to as *Actinomyces p.*, has been isolated from pox lesions on sweet potatoes. This fungus has been found pathogenic on fleshy roots and cut slices of sweet potato, on emerging root-points and rootlets from fleshy roots as well as on the primary root, and on rootlets and stems of sweet-potato sprouts. Resulting lesions are similar to pox lesions occurring on this host under field conditions. Cytological study has shown this same fungus to be present in naturally as well as artificially produced pox lesions on sweet potato.

Actinomyces p. was found pathogenic on cut slices of white potato, beet, and turnip but gave negative results on carrot and dahlia.

Actinomyces p. and *A scabies* were shown to be different from a cultural standpoint, but were pathogenic on cut slices as well as rootlets of sweet potato and white potato.

Cultural and inoculation studies demonstrated that *Actinomyces p.* is different from *A. poolensis*, since the latter fungus was not found to be pathogenic on sweet or white potatoes.

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EXPLANATION OF PLATE

A. Sweet potato slices in sterile agar plates which have been inoculated with *Actinomyces p.* and kept at different temperatures for varying periods. Reading left to right: 33° C. for 7 days, 33° C. for 4 days, and room temperature (20° C.) for 4 days.

B. White potato slices in sterile agar plates which have been inoculated with *Actinomyces p.* and kept at temperatures as described in A for sweet potatoes.

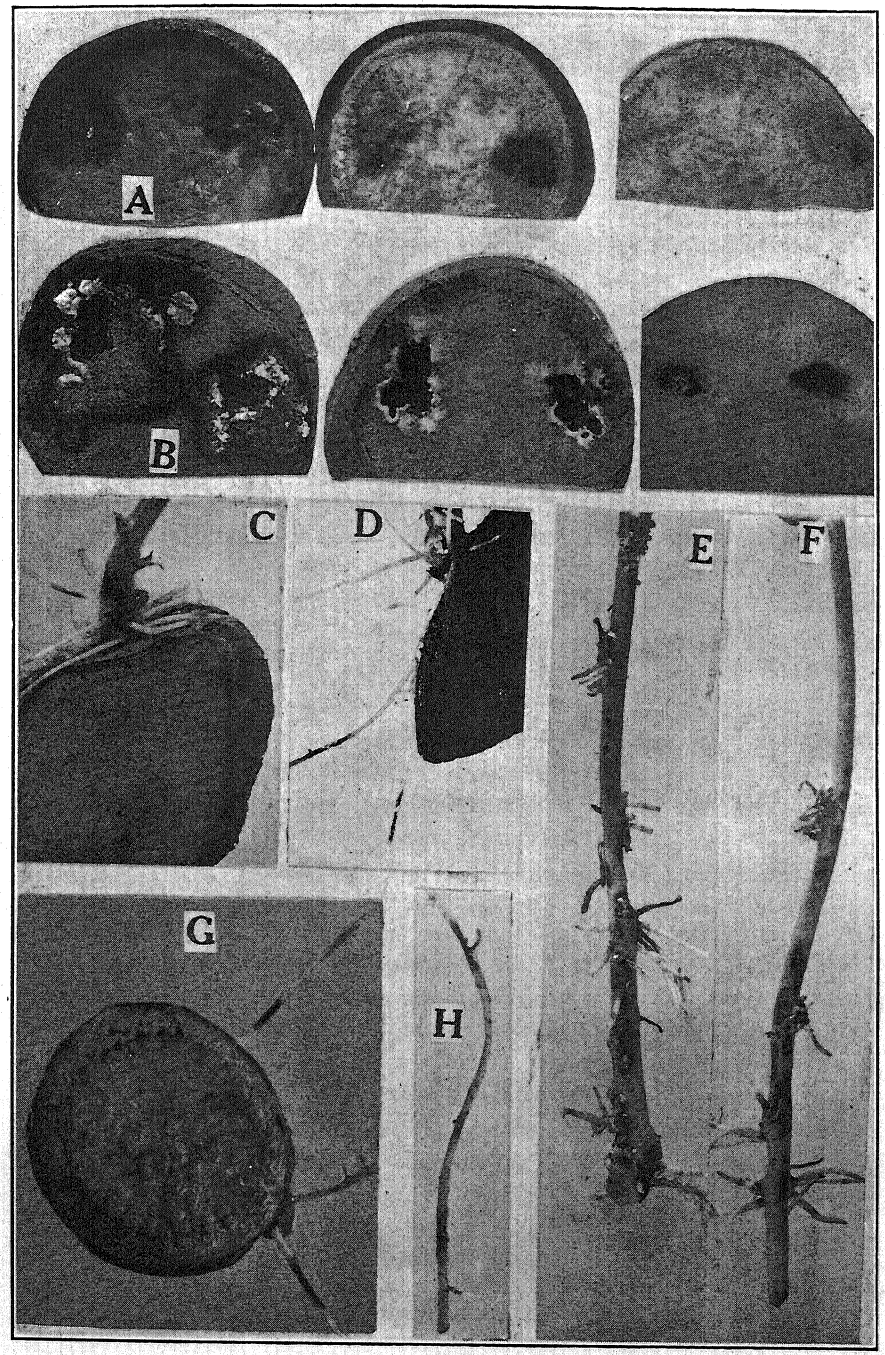
C. Blackened lesions on the base of the sprout and rootlet of white potato inoculated directly with *Actinomyces p.* Inoculation made Feb. 16 and photographed Feb. 22, 1927. Sprouted at room temperature in sterile agar plates.

D. Blackened lesions on rootlets of white potato inoculated directly with *Actinomyces scabies.* Inoculated Feb. 16, 1927, and kept at room temperature for 6 days. Sprouted at room temperature in sterile agar plate.

E and F. Sweet potato sprouts grown under sterile conditions and immersed for three minutes in an inoculum consisting of macerated colonies of *Actinomyces p.* (see text). The sprouts were then removed and placed in sterile beakers with moistened cotton for a substratum. Beakers were plugged at mouths with cotton. The sprout illustrated in fig. E was inoculated Mar. 14 and photographed April 4, 1927; the sprout illustrated in fig. F was inoculated Mar. 25 and photographed Apr. 4, 1927. The secondary root system had been cut off (before photographing) in order to illustrate the primary root lesions.

G. Blackened lesions on rootlets of sweet potato inoculated directly with *Actinomyces p.* sprouted at room temperature in sterile agar plate. Inoculations made Jan. 28 and photographed Jan. 31, 1927.

H. Typical blackened lesions on rootlet cut off of sprout that was immersed for three minutes in an inoculum consisting of macerated colonies of *Actinomyces p.* The


A
B
C
D
E
F
G
H

MONOGRAPH OF THE GENUS PESTALOTIA DE NOTARIS[1,2]

PART I

E. F. GUBA

INTRODUCTION

My interest was directed to the genus *Pestalotia* in the autumn of 1922 by Dr. F. L. Stevens, who had returned to the University of Illinois after several months of botanizing in Hawaii. His collection of fungi contained several specimens of *Pestalotia* which he assigned to me for determination. The volumes of Saccardo's "Sylloge Fungorum" and an excellent botanical library giving access to other compilations and to type descriptions were available for this purpose; nevertheless the specimens could not be accurately identified. The difficulty lay with the indefinite, inaccurate, and carelessly prepared type descriptions. As a result, serious errors in classification have crept into the literature. The general lack of understanding of the species has emphasized the urgent need of their critical study. This first contribution presents a critical study of 46 described species, of which 30 are retained as distinct. It also presents a review of our knowledge of their biologic relationships.

HISTORY OF THE GENUS

The genus *Pestalotia* was created in 1839 by Dr. J. de Notaris (57, p. 80–81) and named in honor of his friend Dr. Fortunato Pestalozza. It was established on the basis of the fungus *Pestalotia pezizoides* de Not. occurring on dead canes of *Vitis vinifera*. The genus was described as follows:

"Sporidia pedicellata pluriseptata, articulo supremo in setas divergentes soluto, infero mutico, vel uni-bi setosa, stromata gelatinoso juncta, ex epidermide erumpentia, demum effusa. Fungilli sub-epidermide ramorum emortuorum nascentes demum liberati, acervula convexa tuberculiformia, in sicco depressa collapsa, sub pezizoidea, sporidia atroinquinantibus."

The fruiting structure was regarded as an acervulus (Plate IV, A). Being seated on a stroma above the epidermis and resembling the outline of an apothecium, de Notaris assigned to it the very appropriate name

[1] The writer is gratefully indebted to Dr. C. W. Dodge, of Harvard University, for helpful suggestions and criticisms in the preparation of this manuscript and for permission to use the facilities of the Farlow Library and Herbarium.

[2] Printed with the permission of the Director of the Massachusetts Agricultural Experiment Station.

Pestalotia pezizoides. The description and illustration of the type of *Pestalotia pezizoides* were prepared carefully enough and sufficiently in detail to offer no chance of error in the identification of this species. The fungus is widely distributed and has been collected generally in Europe and North America, where it is richly represented in exsiccati collections and herbaria.

The first addition to the genus was made by Desmazières in 1840 as *Pestalotia guepini* Desm. on Camellia leaves. This species has 5-celled spores, but is similar to the type of the genus in that the end cells of the spores are hyaline and the apical cell is setulate. The fruiting structure, however, is typical of an acervulus (Plate IV, C.) Desmazières' description proved so indefinite that this species has never been clearly understood. Being the earliest of the 5-celled forms to be reported, its name received cosmopolitan application, a practice which later resulted in innumerable errors in classification. *Pestalotia funerea* Desm., created in 1843, shared a similar fate.

From 1840 to 1880 many new species were added to the genus, the majority of which have 5-celled spores. Saccardo (Syll. Fung. 3: 784. 1884) created the subgenus *Eu-Pestalozzia* for those forms having spores with hyaline end cells, colored median cells, and two or more apiculate setae; *Monochaetia* for those with similar spores but uni-setulate; and *Pestalozzina* for forms with totally hyaline conidia. This arrangement was accepted by Voglino (87) in his compilation of the genus which appeared in 1885. He recognized 89 species, of which 62 belonged to the sub-genus *Eu-Pestalozzia* and 19 to *Monochaetia.* Saccardo (Syll. Fung. 11: 580. 1895) elevated the sub-genus *Pestalozzina* to generic rank and Allescher (Rabenhorst, Kryptog. Fl. I. 7: 665. 1902) did the same for *Monochaetia,* while *Pestalotia* was restricted to the sub-genus *Eu-Pestalozzia.* Höhnel (39) transferred *Monochaetia* to *Hyaloceras.* Diedicke (24) approved of this transfer. Höhnel (40) later, in his system of the Fungi Imperfecti, disregarded *Hyaloceras* and retained *Monochaetia.*

Klebahn (43) divided the genus *Pestalotia sensu strictiore* into sections determined by the number of spore cells as follows:

Quadriloculatae—4-celled spores, two of the cells colored and two hyaline; *e.g., P. stevensonii.*

Quinqueloculatae—5-celled spores, three cells colored and two hyaline; *e.g., P. funerea* and *P. versicolor.*

Sexloculatae—6-celled spores, four colored and two hyaline; *e.g., P. pezizoides.*

This arrangement has been adopted in the text of this paper.

A large amount of literature of a controversial nature exists concerning the pathogenicity of forms of *Pestalotia.* Wenner (89), Bernard (3, 4, 5, 6,

7), Spaulding (74), Brown (12), Tubeuf (82, 83, 84), Laubert (50), Böhm (10), Hennings (38), and others have attributed pathogenic capabilities to certain species, while Doyer (25), Münch (56), Fischer (28) and others regarded them as saprophytes. Doyer (25) denied the pathogenicity of every species reported to be parasitic but her evidence is inconclusive. It appears from a review of the literature that the parasitic nature of many forms has been based upon the constant presence of the fungus on diseased parts of plants rather than on positive evidence of infection obtained by experimental methods. In her selection of material for infection purposes and for determining pathogenic relationships, Doyer disregarded the probable restriction of forms to their hosts. Her failure to infect plants with forms having no biologic relationship to them offers no reasonable basis for accepting her statement of the non-parasitic nature of such species.

La Rue and Bartlett (49) recognized 35 distinct strains within the nominal species of a form identified incorrectly as *P. guepini* Desm. The 35 strains were capable of precise definition along morphological lines into 14 groups. These were obtained from coconut palm, oil palm, betel-nut palm, rubber, and tea, and were not confined to any particular host. La Rue (48) showed that selection is totally ineffective in establishing distinct lines within pure strains of *P. guepini*. Aberrant spores produced normal progeny, while mutations giving rise to lines significantly different from parent lines rarely occurred. These workers accepted the view that the descriptions of the nominal species of *Pestalotia* are generally in no degree diagnostic. They regarded forms obtained from rubber, coconut, tea, oil palm, and betel-nut as the same species. The refined biometrical methods which La Rue and Bartlett have emphasized in arriving at their conclusions seem unnecessary for the classification of species.

Doyer emphasized cultural characters and biometric differences in distinguishing forms, but the study of types is ignored and her knowledge of species is based on existing literature and the carelessly prepared type descriptions. Reports of the occurrence of the same forms on a wide variety of plants have been accepted too freely and her incorrect designation of forms is open to criticism.

The spelling of the generic name received modification from *Pestalotia* to *Pestalozzia* since its inception. Corda (Icones Fungorum 5: 34: 1842) apparently was the first to use the modified spelling. Fairman (27) has shown that *Pestalotia* is both as correct and euphonious as *Pestalozzia*. Such being the case, the original spelling will be used.

MORPHOLOGY

Fruiting Pustules

It is recognized that the genus *Pestalotia* has no typical fruiting struc-

rotted grape berries. The spores were formed within a mass of false tissue and emitted through a narrow ostiole. Wolf believed that the generic description should be modified to include range of types from the flat surface on which spores are formed without pseudoparenchyma to those which form well defined pycnidia. Leininger (52) obtained four types of fructification among forms from *Echeveria* and *Mesembryanthemum* which he called *P. palmarum*. These are as follows: (*1*) Free spores on mycelial threads in liquid media; (*2*) spores on open acervuli and on a loose parenchymatic stroma in liquid media; (*3*) spores in acervuli covered by pseudoparenchyma on solid media; (*4*) spores in pycnidia on solid media.

Leininger found spore bodies in nature with a covering membrane and cavities completely enclosed with hyphae which in basal portions formed a more or less distinct pseudoparenchyma. Klebahn (42), Lagerberg (47), Sartory and Bainier (2, 68), Fischer (28), Doyer (25), and Bertus (8, 9) obtained in culture one or more types of fructification reported by Leininger. Archer's (1) study of the development of the spore bodies of *P. guepini* and *P. palmarum* in culture showed that they originate from a true pycnidium and that cavity formation is like that in the genus *Sphaeropsis*. Klebahn stated that the genus was incorrectly placed in the Melanconiaceae and that its inclusion among the Sphaerioidaceae-Phaeophragmiae close to the genus *Hendersonia* would be more proper. Previous to Saccardo's "Sylloge Fungorum" the fruiting structure of *Pestalotia* was considered a receptacle [Bornorden (11, p. 220 and 228), Frank (31, p. 404 and 32, p. 440), Corda (19, p. 141), Léveillé (53)], in consequence of which the genus was placed with genera many of which are now listed among the Sphaeropsidales. Notaris and Desmazières believed that a receptacle was lacking, while both Montagne and Léveillé observed a covering membrane.

Elenkin and Ohl (26, pp. 94–100) claimed that *P. malorum* E. & Ohl as well as *P. hartigii, P. disseminata,* etc., because of the formation of pseudopycnidia, would be more properly placed in a new genus *Pseudopestalozzia* in the group Pseudosphaerioidaceae Höhnel. Höhnel (40) disposed of the genus in the Gymnostromaceae. Both Höhnel (40) and Potebnia (64) have devised new schemes for classifying the genera of the Fungi Imperfecti, but Gäumann (33, pp. 585–587) and Doyer (25) consider Saccardo's system preferable, as modified by Lindau (Engler and Prantl. Die Natürlichen Pflanzenfamilien. Pilze I. 1: 347–523, 1900), Allescher (Rabenhorst's Krypt. Fl. Pilze. I, 6: 1–1016, 1901; and 7: 1–1072, 1903), and Diedicke (Kryp. Fl. Mark. Brand. IX. 7: 1–962, 1915). Since the fruiting structure of *Pestalotia* may be either an acervulus or a pycnidium the genus may belong to both the Sphaeropsidales and Melanconiales. A simple method of classification would be to place together all setulate spore forms now in the Sphaeropsidales and Melanconiales and to arrange the genera according to form, color, and structure of the spores.

Conidia

The genus is familiar to students wholly by the character of the conidia. These are fusiform in shape, 3-, 4-, or 5-septate with guttulate, colored median cells and hyaline end cells. The spore is supported at the base of the inferior hyaline cell usually by a simple stalk. The apical, hyaline cell is crowned with a crest of 2 or more simple or branched setae. Specimens of *P. pezizoides* in the group Sexloculatae Klebahn reveal an occasional spore with 6 setae. In the 3- and 4-septate spore groups, abnormalities in the number of septa occur. Likewise a variable number of setae on spores from the same specimens may occur, but no specimens have come to my attention whose disposition in either *Monochaetia* or *Pestalotia* required the least hesitation. The elevation of *Monochaetia* and *Eu-Pestalozzia* to generic rank was done in recognition of this fact. Doyer's (25) opinion that they should be combined as one genus is incorrect. The presence of aberrant spores should offer no confusion in classification as they are always negligible in number.

The color intensities of the median cells of the spores have been used as a character for differentiating species. According to Saccardo's "Chromotaxia," the median cells may be olivaceous, umber, or fuliginous. All the median cells may be colored alike or the upper two may be darker than the lower. Doyer arranged the 5-celled spore forms into three groups with spore-color characters as follows: (*1*) *funerea*—three dark middle cells, the lowest the brightest; (*2*) *guepini*—three bright olive colored cells, equally colored; (*3*) *versicolor*—dark band at septa dividing upper two colored cells, lowermost colored cell the brightest. Color contrasts of the median cells, therefore, are considered useful in differentiating species.

The setae never taper to a point, but knobbed setae have been noted by Voglino (87) for *P. clavata* Cke. & Ellis, by Sawada (69), Butler (13) and Bertus (8, 9) for *P. theae* Saw., and by Bertus for *P. palmarum* Cke., and I have found them typical of other species. Lacroix (46) reported that, in the case of *P. funerea* Desm. and *P. choenostroma* Lacr., the rudimentary setae terminate by a slight swelling which later disappears and that the walls of the filaments never taper but remain parallel. However, knobbed setae are constant with certain species. Such species usually have knobbed pedicels. Infrequently one or more of the setae originate at the margin of the uppermost septa of the spore. Graves (35) found such a condition existing among spores of a species which he designated *P. funerea* Desm., and Voglino (87) reported this characcter for *P. montellica* Sacc. & Vogl. Branched setae are typical of many species, notably *P. guepini* Desm. and *P. pezizoides* de Notaris. These spore characters are extremely useful in differentiating species.

A close relation exists between *Pestalotia* and *Hendersonia,* for according to Lagerberg (47) cultures of *P. hartigii* yielded some conidia similar to those of *Hendersonia* and some resembling those of *Monochaetia* and *Coryneum.* According to Massee (54), *P. guepini* cultivated on bread produced conidia without setae exactly like those of *Hendersonia theicola* Cke., but when conidia of the latter were transferred again to prune decoction only Pestalotia conidia were produced. These he regarded as abnormal forms of *P. guepini.* On the other hand Speshnev (75) failed to obtain setulate conidia in cultures of *Hendersonia theicola* therefore concluding that *P. guepini* and *Hendersonia theicola* were distinct. Crié (20) found setulate spores resembling *Pestalotia* among the conidia of certain forms of *Hendersonia.* Spores resembling those of *Pestalotia* and *Diplodia* were found in the same receptacle. These variations in spore form suggest that the species are in a plastic condition, but La Rue (48) found that selections according to progeny and visible characters for several generations failed to establish any differences and that selection was ineffective in establishing distinct lines within pure strains. Mutations giving rise to lines significantly different from parent lines infrequently occurred.

Other Spore Forms

Sartory and Bainier (2, 68), Wenner (89), and Doyer (25) found chlamydospores in cultures. The spores are borne in long chains which finally fall apart. The cells of these spores are capable of germinating and again forming mycelium and conidia. Wenner was the first to show the existence of a second spore form in the genus. According to Wenner, chlamydospores may arise in either of two ways as follows: (1) any cell or several cells together in a hypha may round out and become fairly thick-walled spores; (2) the entire distal portion of a hypha may form consecutive, short, cylindrical spores with fairly thick walls. These, according to Wenner (89), are similar to oidia.

Höhnel (41) reported both *Ceratostoma vitis* Fuckel and *P. truncata* Lév. on *Humulus lupulus* in Krieger, Fung. Sax. 580 and assumed that the former was the ascigerous stage of the latter. This is the only report of the ascigerous stage of a *Pestalotia.* This connection seems very doubtful, especially in the absence of study of either form in pure culture. Species of *Pestalotia* existing as saprophytes on dead, decaying parts of plants are frequently associated with other fungi. The latter cannot be considered related because of mere association.

Taxonomy

PESTALOTIA de Notaris, Microm. Ital., Dec. II. Mem. R. Accad. Sci. Torino II. 3: 80–81. 1839.

Fruiting bodies black, carbonaceous, either acervuli with or without stromata or pycnidia. Spores fusiform, 4- to 6-celled of which the exterior

cells are hyaline and the intermediate cells dark-colored, guttulate; superior hyaline cell with a crest of two to several simple or branched setae, inferior hyaline cell supported by a simple or branched pedicel.

Section Sexloculatae Klebahn, Myc. Centralbl. **4**: 19. 1914.

Acervuli seated on a stroma composed of erect, septate, branching or simple filaments, pezizoid. Spores fusiform, 6-celled, interior cells colored, exterior hyaline; superior cell crowned with a crest of two or more simple or branched setae, inferior cell tapering into a branched stalk. The type is *Pestalotia pezizoides* de Notaris on dead canes of *Vitis vinifera*, Milan, Italy.

1. PESTALOTIA PEZIZOIDES de Notaris, Microm. Ital. Dec. II. Mem. R. Accad. Sci. Torino II. **3**: 80–81. 1839; Pirotta, Arch. Trienn. Lab. Bot. Critt. Pavia **2**: 196–198. 1878; Sacc. Syll. Fung. **3**: 789. 1883.

   P. compacta B. & C., Grevillea **2**: 155. 1874; Sacc. Syll. Fung. **3**: 795. 1884.

   P. ramosa D'Almeida, Rev. Agr. Soc. Sci. Agron. Portugal **1**: 23–26. 1903; Sacc. Syll. Fung. **18**: 481. 1906.

Fruiting bodies definite, scattered or gregarious, often confluent, erumpent, borne on a thick gelatinous stroma, discoid, pezizoid or subglobose, 225–600 × 100–300 μ, protruding well above the epidermis (Fig. 1, b).

Spores long fusoid, equilateral, sometimes slightly curved, tapering at the ends, 6-celled, 27–36 × 6–9 μ; intermediate cells olivaceous, slightly constricted at the septa, 19–26 μ long; setae short, 2 to 5, branched or simple, 6–18 μ, recurved, rarely with a solitary branched seta and 12–31 μ; pedicel simple or forked, 2–10 μ, sometimes 5–22 μ, slender (Plate —, A and B).

On canes of *Vitis vinifera, V. riparia* and related species.

Specimens examined: Michener Fungi. **19**: 82, 2790, N. Garden, C. Co., Pa., sub *P. compacta* B. & C., in Myc. Coll. U. S. D. A., and similar specimens in Curtis Herbarium, *6030,* at Harvard and Berkeley's Herbarium at Kew, wrongly cited on stems of *Humulus lupulus;* Ellis, No. Amer. Fung. *35,* on grape vines; Ravenel, Fung. Amer. Ex. *256* on twigs of Vitis, Aiken, So. Carolina, 1877; Barth., Fung. Col. *1631,* on dead *Vitis riparia,* Rooks Co., Kans., Nov. 15, 1901 and March, 1897; on *Vitis sp.*, Marshall Hall, Md., V. K. Charles, 7–6–06, Myc. Coll. U. S. D. A. sub *P. ramosa* D'Almeida.

The acervulus is supported by a dense thick stroma which arises above the epidermis and in habit resembles the apothecium of a *Peziza.* The spores are exposed on flat or concave stromatic cushions. They are 6-celled and possess other characters different from our present conceptions of the genus. Pirotta published a very good account of the fungus. Cooke

(Nuovo Giorn. Bot. Ital. **10**: 27. 1878) regarded *Hendersonia longipes* B. & C. synonymous with *P. pezizoides*. According to the type description of *H. longipes* B. & C., this form has 3-septate spores and no setae.

In 1903 D'Almeida created the species *P. ramosa* on canes of *Vitis vinifera*, Chamusca, Portugal. The figures accompanying the description show clearly that the fungus is identical with *P. pezizoides* de Notaris. Duplication of this species was due apparently to confusion in the literature regarding the number of septa in the spores. De Notaris specified and figured 5-septate spores although he allowed for an exception when he stated "sporidia . . . plerumque 5 septata." Saccardo (Syll. Fung. **3**: 789. 1883) and Allescher (Rabenhorst, Kryptog. Fl. Deutschl. I. **7**: 693. 1902) stated that *P. pezizoides* has 5-celled conidia. Voglino (87) regarded it as a 4- to 5-septate form. Desmazieres (Ann. Sci. Nat. Bot. II. **19**: 336. 1843) mentioned 5 septa. Klebahn (43) observed 6-celled conidia in specimens of *P. pezizoides* in Barth., Fung. Col. *1631*. My study of many exsiccati specimens confirms the 6-celled character of the spores of this species.

The name *P. compacta* B. & C. was apparently intended for another species, for the type description by Berkeley mentioned 3-septate spores and the source of the material as South Carolina. The original material was acquired by Michener in Pennsylvania. Michener gave specimens to Curtis in South Carolina and Curtis shared his material with Berkeley. The pencil drawing of the fungus accompanying the type material from Kew, presumably by Berkeley, shows 5-septate spores. Either an error was committed in the designation of the host by the collector or Michener confused this material with something else. Dr. E. C. Jeffrey of Harvard University, to whom material was submitted, reported that the host is undoubtedly a species of *Vitis*, not *Humulus lupulus*. The fungus is identical with *P. pezizoides*.

Section Quinqueloculatae Klebahn, Myc. Centralbl. **4**: 3. 1914.

Pustules subepidermal, erumpent, typical of acervuli or pycnidia; spores fusiform, 5-celled, interior cells colored, exterior hyaline: superior cell with a crest of two to several setae: inferior cell supported by a simple pedicel. The type is *Pestalotia guepini* Desm. on leaves of *Camellia japonica*, Angers, France.

2. PESTALOTIA GUEPINI Desm. Ann. Sci. Nat. Bot. II. **13**: 182–184. 1840. pl. 4, fig. 1–3; Sacc. Syll. Fung. **3**: 794. 1884.

Coryneum camelliae Massee. Grevillea **20**: 8. 1891. Kew, Bul. Misc. Inform. 138: 106–109. 1898.

Pestalotia karstenii Sacc. & Syd. Sacc. Syll. Fung. 14: 1030. 1899.

P. inquinans Karst. Hedwigia **30**: 301. 1891; Sacc. Syll. Fung. **10**: 487. 1892; not Cke. & Harkn. Grev. **12**: 94. 1884.

Spots about 1.5 cm. in diameter or variable in area and shape, ash gray on the upper surface, sometimes with a definite dark or reddish brown border; frequently the dead areas are confined to the margins of the leaves, or the entire leaf may be colored brown and covered with the fungus (Fig. 1, a).

Fruiting bodies on grayish papery spots or scattered over the entire surface of the leaf, punctiform, black, 90–170 μ diam., numerous, amphigenous,

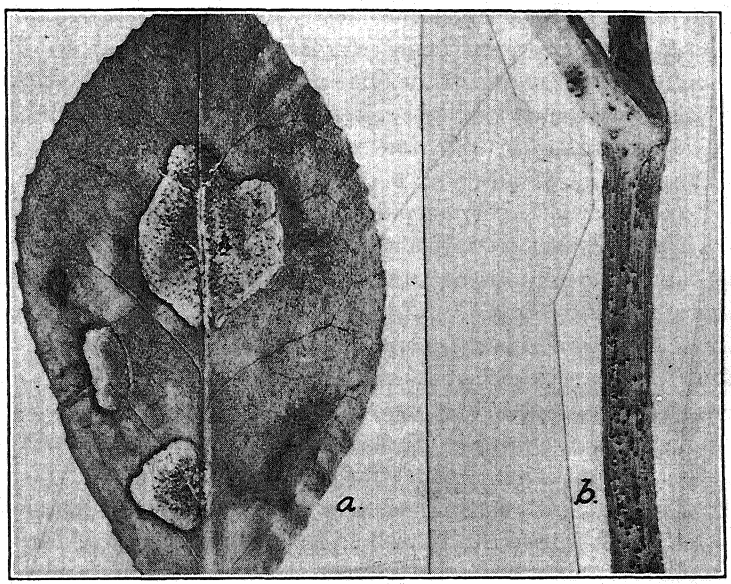


FIG. 1. a. *P. guepini* Desm. on leaf of *Camellia japonica*.
b. *P. pezizoides* de Notaris on cane of *Vitis vinifera*.

globose-lenticular, rupturing the epidermis, the black contents spreading over the surface of the leaf.

Spores erect, 14–21 μ, elliptic-fusoid, 5-celled, hardly constricted at the septa; end cells hyaline, short-conic, not tapering, sometimes rather inconspicuous; median cells olivaceous, guttulate, 10–14 × 5.5–6.6 μ; setae 1, 2, 3, or rarely 4, sometimes with a small inconspicuous knob at the apex, 10–24 μ, divergent, commonly branched; pedicel short, straight, often deciduous (Plate IV, C and D).

On stems and leaves of *Camellia japonica*, usually associated with pale colored spots (Fig. 1, a).

Specimens examined: (on leaves), Desmazières, Pl. Crypt. de France **22**: 1084, Angers, France, Guepin; Roumeguère, Fung. Sel. Gall. Ex. *315*,

Hort. Lotharingiae and Alsatiae; Thüm., Fl. Aust. Ex. *1070,* Bohemia, 1873; Ellis, No. Amer. Fung. *256,* Westchester, Pa., in greenhouse, G. Martin, Sept. 1880; Briosi and Cavara, I Funghi Par. VI, *150,* Pavia, 1890; Rab., Fung. Eur. Ex. *2352,* in Hort. Bot. Neapolitano, Cesati; Tuskegee Inst. Alabama, 3–7–25, G. W. Carver; Alabama, 3–17–12, F. A. Wolf, Myc. Coll., U. S. D. A. sub *P. inquinans* Cke. and Hark.; Washington, D. C., in greenhouse, 5–20–07, W. A. Orton, Myc. Coll., U. S. D. A. sub *P. camelliae* Pass.; Pass Christian, Mississippi, 1–29–16, E. Martin, Myc. Coll., U. S. D. A.; Herb. J. A. Stevenson *1304,* Oneco, Fla. 2–10–21; Washington Insp. House, P. I. *5099* U. S. D. A., Nantes, France; Cooke F. Brit. Ex. *625,* 2nd Ed.

P. guepini has been cited incorrectly on a wide range of plants. Speshnev (75) reported it on *Camellia japonica, Thea, Citrus, Magnolia* and *Rhododendron* in the region of the Black Sea. The form on *Thea* which he studied was described as having 4-celled spores. Saccardo (Syll. Fung. **3**: 794. 1884) reported *P. guepini* on *Camellia, Rhododendron, Citrus, Lagerstroemia, Magnolia, Smilax* and *Amygdalus.* La Rue's (48) concept of *P. guepini* is that of a polymorphic fungus containing many strains causing the gray blight of tea, leaf spot of coconut, betel-nut palm, African oil palm, para rubber, and other plants, but this view is not acceptable in the light of studies by Butler (13), Bertus (8, 9), and Archer (1), which have shown that the forms from tea, palm, and *Camellia* are distinct species. According to my studies, *P. guepini* is restricted to *Camellia.*

Massee (54) regarded *Hendersonia theicola* Cke. (Grev. **1**: 90. 1872) synonymous with *P. guepini.* Massee, like Speshnev, illustrated and described 4-celled spores but his illustration of the leaf spot is typical of the Camellia disease. He cultured the fungus from *Camellia* on bread and found some spores wholly devoid of bristles, similar to those of *Hendersonia theicola* on tea leaves; but when transferred to plum juice gelatin these spores produced normal conidia. Speshnev disagreed with Massee because cultures of this *Hendersonia* failed to obtain *Pestalotia* conidia. Massee also failed to offer proof that *Pestalozzina camelliae* Pass. (Rev. Myc. **9**: 146. 1887) is identical with *P. guepini* Desm. The type description is distinctly that of a *Pestalozzina.*

The type specimen of *P. guepini* Desm. in Desmazières Pl. Crypt. de France **22**: 1084, was inaccurately described and illustrated but the characters of the original specimens agree with the description of *P. inquinans* Karsten (Hedwigia **30**: 301. 1891) = *P. karstenii* Sacc. & Syd. (Sacc. Syll. Fung. **14**: 1030. 1899) occurring on living leaves of *Camellia.* The name *P. karstenii* was substituted for *P. inquinans* to avoid confusion with another *P. inquinans* Cke. & Hark. (Grev. **12**: 94. 1884) on dead leaves of *Eucalyptus.*

According to Klebahn (43) the conidia of *P. guepini* in Roumeguère, Fung. Sel. Gall. Ex. *315,* do not agree with those of the type illustrated by Desmazières. This specimen has short elliptic-fusoid spores occasionally with forked setae in contrast to the long, narrow, fusoid spores with simple setae illustrated by Desmazières. Massee (54) illustrated spores of *P. guepini* with 3–4 simple setae but with only 3 septa. Voglino (87) figured spores of *P. guepini* similar to Desmazières' illustrations. His description of the fungus is accompanied by reference to the following exsiccati; Rab. Fung. Eur. Ex. *2352,* on leaves of *Camellia japonica:* Thüm. Fung. Aust. Ex. *1070,* on leaves of *Camellia japonica:* Ravenel, Fung. Amer. Ex. *553,* on leaves of Persimmon and Cooke Fung. Brit. Ex. *625,* 2nd Ed. on leaves of *Camellia japonica.* Ravenel, Fung. Amer. Ex. *553,* on persimmon leaves labelled *P. guepini* is a different species.

Karsten's description of the setae of *P. karstenii* Sacc. & Syd. (*P. inquinans Karsten*) is as follows: "Ciliis apicalibus 2, rarissme 1 vel partitione 3, divergentibus, sub-aequalibus, hyalinis, 15–35 μ longis." Other differences stated here are: "*A Pestalozzia guepini* Desm. Camelliam incolente, acervulis, macula pallescente insidentibus, mox superficialibus nex non cilus paucioribus discrepat." Allescher (Rabenhorst, Kryptog. Fl. Deutschl. I. **7**: 685. 1902) and Klebahn (Myc. Centralbl. **4**: 7–9. 1914) regarded *P. karstenii* a synonym, and my study of the type confirms this view. Desmazières figured spores of the type with pointed setae, but Lacroix (46) never observed such setae in his studies in spore development, and I have never encountered them among any forms. Since the fungus was carelessly described, Karsten was unable to reconcile his fungus with the description, whereupon he created another species.

Massee (54) proved by artificial inoculation that *P. guepini* is a parasite and the cause of a common leaf spot of *Camellia.*

3. **Pestalotia vaccinii** (Shear) Guba, n. sp.
   P. guepini Desm., var. *vaccinii* Shear, Bul. Torrey Bot. Club **29**: 456–457. 1902.

Acervuli hypophyllous, subepidermal then erumpent, black, coalescing freely, sooty at maturity, 100–250 μ diam.

Spores rather long fusoid, straight or somewhat inequilateral, 5-celled 21–25 × 5.5–6.6 μ; the three central cells guttulate, 15–16 μ, the two upper colored darker than the lower; exterior cells hyaline, basal long conic, apical long cylindric with 3, rarely 4 setae, 22–30 μ; pedicel 4–14 μ (Fig. 4, *12*).

On dead leaves of *Vaccinium macrocarpon,* Parkdale, New Jersey, Sept. 1901, C. L. Shear.

While this species may have some resemblance to Desmazières' illustration of *P. guepini* (Ann. Sci. Nat. Bot. II. **3**: 182, pl. 4, fig. 1–3, 1848),

it differs decidedly from the type in Desmazières Pl. Crypt. de France **22**: *1084*, and from other exsiccati specimens. This fungus is sufficiently characteristic to deserve recognition as a distinct species.

According to Shear (72, pp. 38–39) *P. vaccinii* is generally distributed in cranberry-growing regions, specimens having been obtained from West Virginia, New Jersey, Wisconsin, Massachusetts, and New York. Leaves and fruit are affected, but the fungus does not attack fruit with sufficient frequency to cause much loss. It has been found on leaves which show no external signs of disease. N. E. Stevens (77) considered this fungus as one of the many organisms causing decay of cranberries. He recovered it from rotted green berries and from healthy green berries as early as the middle of July.

4. PESTALOTIA CESATII Rabenhorst, Fungi Europaei, *1932*. 1875.

Fruiting bodies largely hypophyllous, scattered, at first subepidermal, then erumpent, rupturing the epidermis usually in a stellate manner and exposing the black pustules, 96–160 μ wide.

Conidia 5-celled, broad fusiform, erect, rarely curved, slightly constricted at the septa, 24–26.5 μ; 3 median cells olivaceous, equally colored, 16–19 μ × 8.5–9.5 μ; end cells hyaline, the basal short, conic, or obtuse, supported by a coarse pedicel, 4–12 μ; apical cell short, conic, bearing a crest of usually 5 short, erect or acutely divergent setae, 7–19 μ, frequently knobbed at the extremities and somewhat unequal in length (Fig. 3, *6*).

On moist leaves of *Podocarpus latifolia* in Botanic Gardens, Naples, Cesati, in Rab., F. Eur. *1932*.

5. PESTALOTIA FUNEREA Desm., Ann. Sci. Nat. Bot. II. *19*: 335–336. 1843; Sacc. Syll. Fung. **3**: 791. 1884; Klebahn, Myc. Centralbl. **4**: 5. 1914.

P. conigena Lév., Ann. Sci. Nat. Bot. III. **5**: 285. 1846; Sacc. Syll. Fung. **3**: 792. 1884; Klebahn, Myc. Centralbl. **4**: 6. 1914.

P. abietina Roumeguère, Fung. Sel. Gall. Ex. *735*. nomen nudum.

Pustules amphigenous, 100–250 μ in diameter, usually scattered, sometimes confluent, black, subepidermal, later erumpent and surrounded by the torn epidermis, on maturity liberating the sooty contents.

Spores clavate or elliptic-fusoid, 21–27 × 7–9 μ, equilateral, 5-celled; median colored cells guttalate, umber, the lower sometimes brighter, 15–17.5 μ, only slightly constricted at the septa, tapering at the lower end; exterior cells hyaline, the apical cell usually small, somewhat inconspicuous, bearing a crest of 3, 4, or 5 but rarely 6 setae, usually simple and erect, 5–20 μ; basal hyaline cell usually long conic supported on an erect pedicel, 2–7 μ (Fig. 3, *8* and *9*).

On leaves, bark, and cones of *Thuja orientalis, T. occidentalis, Retinospora squarrosa, Chamaecyparis spp., Abies lanceolata, Cupressus spp.* and related species. The type fungus was described on dead leaves of *Thuja.* The original material was not accessible but study of specimens on coniferous plants, whose characters agree with Desmazières' type description, leaves no doubt of their identification. The usual presence of 4–5 erect setae, broad fusoid spores, dark olivaceous colored median cells, and the small apical hyaline cell are particularly characteristic of this fungus.

Specimens examined; on *Abies lanceolata* (leaves); Roumèguere, Fung. Sel. Gall. Ex. *735.* Main et Loire, Angers, France. Guepin, sub *P. abietina* n. sp. (*P. guepini* Desm. var. *abietina* Guepin Mss.)

On *Chamaecyparis lawsoniana* (leaves and twigs); Sydow, Myc. Germ. *839.* Tamsel, Brandenburg, Germany, Apr. 1, 1909. P. Vogel; Kabat and Bubak, Fung. Imp. Ex. *739,* Febr. 7, 1911.

On *Cupressus sp.,* Rab. Herb. Myc. Ed. Nov. 1: 63, Brixia, Italy, Cesati sub. *P. funerea* Desm. var. *heterospora*; on *C. macrocarpus* (bark), Ellis, No. Amer. Fung. *634,* California, Harkness.

On *Retinospora squarrosa* (leaves); Sydow, Mycoth. March. *4440,* Bellevue Garten, Berlin, Germany, Oct. 1895, P. Sydow.

On *Thuja orientalis elegantissima* (leaves); Shiloh, N. J. Sept. 9, 1927, White; on *T. occidentalis* (cones), Ravenel Fung. Amer. Ex. *700,* Aiken, So. Carolina; Herb. N. Y. State Mus., Oneida, N. Y., June 8, 1923, H. D. House; Ellis No. Amer. Fung. *527,* Sept. 1880, sub *P. conigena* Lév.; (leaves) Dearness, *3325,* and Barth. Fung. Col. *3535,* London, Canada, Aug. 1911, J. Dearness.

Much confusion exists regarding the identity of this fungus. Specimens on a wide range of host plants have been incorrectly named. The fungus is restricted to plants of the Pinaceae. Both Saccardo and Rabenhorst have listed several hosts in many families and Doyer (25) consequently has stated that the fungus is omnivorous.

P. funerea Desm. and *P. conigena* Lév. are identical. The type of the latter occurs on cones of *Thuja occidentalis* and *Pinus sylvestris.* Léveillé's description is extremely indefinite. All statements of size and color are lacking and the number of septa is either 3 or 4. On the other hand, such characters as "sporis elongatis in stipitem attenuatis—filis 4 pellucidis" and the obtuse apex are suggestive of the similarity of Léveillé's fungus. The early conceptions of *P. conigena* were contradictory. Some mycologists regarded it as a 4-celled spore form, others as having 5 cells. Saccardo (Syll. Fung. **3**: 792. 1884) reported 4 septa with 3 dark median cells, and Klebahn (Myc. Centralbl. **4**: 6. 1914), Voglino (87), and Tubeuf (82, pp. 40–51) adhere to this view. Tubeuf and Voglino have established that the

4-celled spore form in Rabenhorst Fung. Eur. *2462* is not *P. conigena* Lév. and both have described it as a new species, Voglino as *P. abietina*, and Tubeuf as *P. conorum-piceae*. Both of these names are synonymous with *P. stevensonii* Peck. Gleisberg (34) regarded both *P. conigena* Lév. and *P. foedans* Sacc. & Ell. synonymous with *P. funerea*. My study shows that *P. foedans* is a distinct species.

Doyer's interpretation of *P. funerea* is confusing. Without knowledge of the type, and basing her conception of this species on descriptions by Saccardo and Klebahn, she claimed to have isolated this fungus from *Rhododendron* and various conifers. On the basis of descriptions of *P. macrotricha* Kleb., *P. conigena* Lév. and *P. foedans* Sacc. & Ell. furnished by these workers, she assigned forms isolated from *Taxus, Retinospora,* and *Biota* to *P. macrotricha* and those from *Thuja, Chamaecyparis, Juniperus,* and *Pinus* to *P. funerea*. According to my studies *P. macrotricha* is limited to *Rhododendron* and shows no similarity with forms on conifers.

The validity of varieties within this species has been doubted by Gleisberg (34) and Doyer (25). My own studies indicate that the assumed relationship of the numerous described varieties of *P. funerea* is incorrect. It is also evident that this species is not of wide host range. Disposition of the described varieties of the species is reserved for a future paper.

Differences of opinion exist concerning the biology of *P. funerea*. There is some evidence in favor of its parasitic habit. Böhm (10) reported this species as causing the blight of distal shoots of *Thuja menziesii*. He was successful in obtaining infection with spores applied to wounds on living twigs of *Thuja menziesii, Chamaecyparis lawsoniana,* and *Pseudostuga douglasii,* but after five months no spores had developed. The extensive dying of junipers at Eberswalde which Böhm attributed to *P. funerea* is claimed by Danckelmann (21) to be winter killing from temperatures of $-30°$ to $-32°$ C. in the winter of 1892–1893. Gleisberg (34) inoculated living parts of *Thuja occidentalis* with spores of *P. funerea* obtained from bark of the same host but without success, and he concluded that *P. funerea* was a saprophyte which developed only after a change in the chemical and physical nature of the bark.

Spaulding (74) reported a serious needle blight of *Pinus ponderosa* and *P. divaricata* on two-year-old seedlings from which he recovered *P. funerea*. With this inoculum he succeeded in infecting healthy seedlings of *Pinus ponderosa*. A similar disease has been reported from South Africa by Fisher (29) on *Pinus pinaster, P. canariensis, P. longifolia, P. insignis, P. massoniana, Cupressus lusitanica, Chamaecyparis lawsoniana,* and *Casuarina leptoclada*. The organism was identified as *P. funerea* but no proof of its pathogenicity was established. Hartley, Merrill, and Rhoads (37) re-

ported *P. funerea* very common on dead coniferous needles throughout the United States. With cultures obtained from jack and Rocky Mountain yellow pines, inoculations of white pine were without result. Attempts to infect green shoots of *Thuja occidentalis,* some of which had been injured by puncturing or heating, with spores from the same plant failed.

In an earlier paper Hartley (36, pp. 15–18) reported having isolated 4- and 5-celled spore forms of *Pestalotia* from damping-off coniferous seedlings, but that cultures were not sufficiently frequent to indicate parasitism. Smith (73) found *P. funerea* associated with withered distal portions of shoots of *Biota orientalis* in England, and reported a similar phenomenon on species of *Thuja, Cupressus, Chamaecyparis,* and *Juniperus.* He assumed the fungus to be parasitic because Tubeuf (82, pp. 40–51) earlier regarded *P. hartigii* as the cause of the "Einschnürungskrankheit" of forest tree seedlings in Germany. Wenner (89) isolated *P. funerea* from bark and needles of *Pinus strobus* and his inoculation experiments proved conclusively that this species is parasitic under certain conditions, the most important of which is the presence of moisture. The fungus was shown to be capable of attacking leaves and stems of white pine, Norway spruce, and hemlock. A needle blight of white pine very prevalent in Connecticut in 1907 was attributed by Clinton (16, 17) to adverse weather conditions. Studhalter (80) infected artificially the needles of *Pinus radiata* apparently with *P. funerea.* Graves (35) found a *Pestalotia* on needles of withered shoots of Norway spruce, and the manner of the appearance of the disease suggested a parasitic relationship.

Doyer (25) inoculated seedlings of *Pinus sylvestris* and *Picea excelsa* in wounds, in spots injured by heat, and elsewhere with *P. funerea* from *Thuja* and *Chamaecyparis*; with a form from *Taxus, Biota,* and *Retinospora* which she named *P. macrotricha*; with a form from *Thuja* which she named *P. versicolor* Speg.; and with Fischer's strain of *P. hartigii.* But in no case was infection obtained. Negative results were also obtained on the same plants with Brown's strain of *P. scirrofaciens* Brown, *P. monochaetioides* Doyer, *P. virgatula* Kleb., and *P. theae* Saw. Further inoculations with the same forms of a number of conifers under various conditions and at different times of the year failed. The existing differences of opinion of the biology of *P. funerea* which this review indicates justify further study.

6. PESTALOTIA FOEDANS Sacc. & Ellis, Michelia **2**: 575. 1882; Sacc. Syll. Fung. **3**: 792. 1884.

Pustules densely gregarious, elongate and hysteriform, innate erumpent, coalescing freely, rupturing bark in a longitudinal manner, 200–450 × 90–270 μ, twice or more as long as wide, contents blackening the matrix.

Conidia fusoid, 5-celled, 19–24 × 5.5–6.5 μ, equilateral, erect, only slightly constricted at the septa; the dark interior cells 14–16.5 μ, guttulate, the upper two umber, the lower olivaceous; the basal cell prominent, rather broad-conic, the apical small, acute, bearing at its apex a crest of 3, rarely 2 or 4, slender, divergent setae, 10–17 μ; pedicel short, straight, 4–8 μ (Fig. 3, *5*).

On decaying bark of *Chamaecyparis thyoides*. Masses of acervuli and spores cover the affected surfaces.

Specimens examined: Ellis, No. Amer. Fung. *954*, Newfield, N. J., Oct. 1880, Ellis and Harkness, and similar specimens in E. and E., Fung. Col. *81*, and Rab., Fung. Eur. Ex. *2997*.

7. **Pestalotia glandicola** (Cast.) Guba n. comb.
   Robillarda glandicola Castagne, Cat. Pl. Marseille 205, Pl. IV. 1845.
   Pestalotia castagnei Desm., Ann. Sci. Nat. Bot. III. **6**: 64. 1846; Desm. Pl. Crypt. de France **30**: 1490. 1846; Sacc. Syll. Fung. **3**: 793. 1884.

Pustules sub-epidermal, erumpent on maturity, coalescing freely, forming dense black cushions, globose or lenticular, tearing covering irregularly, 160–480 × 160–320 μ.

Spores 5-celled, fusiform, somewhat curved, 22.5–25 μ; median cells dark, stricted at septa dividing the two lower colored cells; end cells rather large, the upper two fuliginous, the lower olivaceous, 15.5–18 × 8.4–9.5 μ, conprominent, the apical conic to cylindric, the basal conic, tapering; setae 3, divergent, 9–19.2 μ; pedicel 2.4–7.2 μ, erect (Fig. 2, *1*).

On dried fallen acorns of *Quercus ilex* from France, in Desm. Pl. Crypt. de France **30**: *1490*. 1846 and Curtis Herbarium at Harvard. The pustules are sub-epidermal. The black contents issue in thick coarse threads and on being moistened spread out around the perforations. Three hyaline, filiform setae shorter than the spores surmount the apical cell. The setae often spread out at right angles and rarely lie down over the spore. Castagne originally described this species as having 3-celled spores, with a central colored cell and hyaline exterior cells. Setae were not mentioned. Type specimens distributed by Castagne bear only spores of *Pestalotia*.

8. PESTALOTIA SPHAERELLOIDES Ellis & Langl., n. sp., Sacc. Syll. Fung. **13**: 268. 1898. nomen nudum.

Acervuli hypophyllous, subglobose, scattered but gregarious in places, like pycnidia, raised, erumpent, circinate, surrounded by the torn epidermis, 100–150 μ wide.

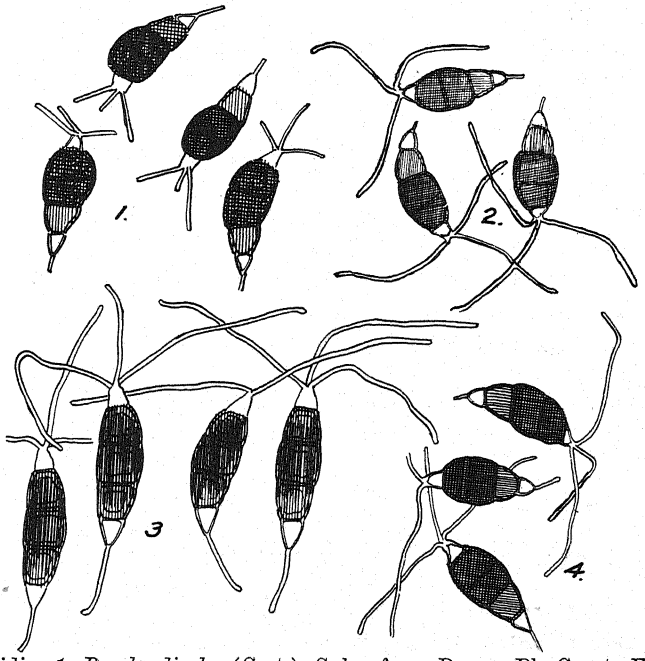


FIG. 2. Conidia: *1, P. glandicola* (Cast.) Guba from Desm., Pl. Crypt. Fr. Fasc. 30, *1490,* on acorns of *Quercus ilex,* sub *P. castagnei* Desm.; *2, P. scirpina* E. and M. from E. and E., No. Amer. Fung. 2d. Ser. *2181,* on dead culms of *Scirpus olneyi; 3, P. macrotricha* Klebahn from E. and E., Fung. Col. *370a,* on *Rhododendron maximum,* sub *P. guepini* Desm.; *4, P. sphaerelloides* Ellis. and Langl. from E. and E., No. Amer. Fung. *2387,* on leaves of *Carya aquatica.* ×750.

Spores 5-celled, erect, equilateral, broad-clavate, constricted at septum dividing the two lower colored cells, 19.5–25.5 μ; median cells dark colored. the upper 2 fuliginous, the lower olivaceous, 15–17 × 8.5–9.5 μ; end cells hyaline, conspicuous, elongate, the apical cell bearing 2 or usually 3 long, widely divergent setae, 19–30 μ; pedicel 4–7 μ (Fig. 2, *4*).

On leaves of *Carya aquatica,* Bayou Chêne, Louisiana, Oct. 1888, A. B. Langlois in E. & E., No. Amer. Fung. *2387.* Distinct pycnidium-like pustules are scattered generally over the lower surface of the leaves.

9. PESTALOTIA CLIFTONIAE Tracy & Earle, Bul. Torrey Bot. Club **22**: 178. 1895; Sacc. Syll. Fung. **14**: 1027. 1899.

Acervuli epiphyllous, punctiform, scattered, erumpent, circular, 120–200 μ wide, seated on orbicular or circular pale or brown spots not exceeding 1 cm. in diameter with definite narrow purple margins.

Conidia obovate, usually curved, unequilateral, 5-celled, constricted at the septum dividing the 2 lower colored cells, 19–24 μ, septa often oblique;

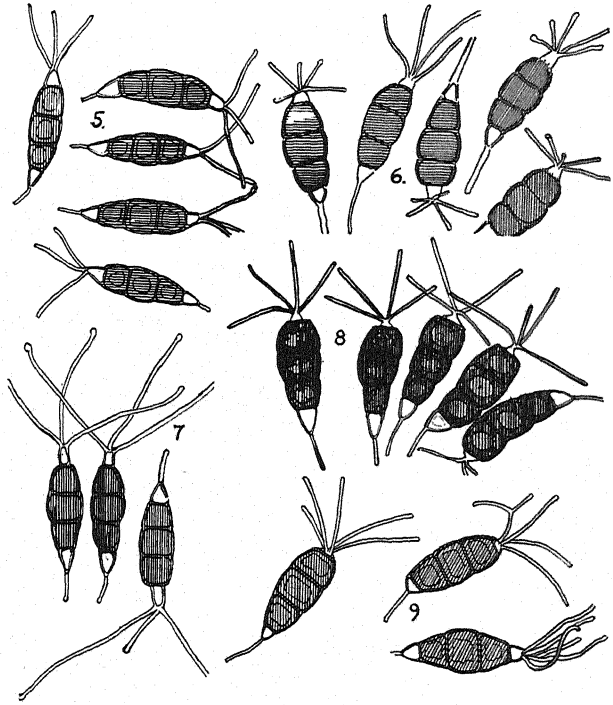


FIG. 3. Conidia: *5, P. foedans* Sacc. & Ellis from Ellis, No. Amer. Fung. *954*, on decaying cedar bark, Newfield, N. J. Oct., 1880, Ellis and Harkness; *6, P. cesatii* Rab. from (Rab.) Fung. Eur. *1932*, on leaves of *Podocarpus latifolia*, Bot. Gardens, Naples. Cesati; *7, P. theae* Sawada from Sydow Fung. Exot. Ex. *146*, on leaves of *Camellia theae*, sub *P. palmarum; 8, P. funerea* Desm. from Ellis, No. Amer. Fung. *527*, on fallen cones of *Thuja occidentalis*, sub *P. conigena; 9, P. funerea* Desm. from Roumeguère Fung. Sel. Gall. Ex. *735*, on *Abies lanceolata*, Angers, France, Guepin, sub *P. abietina* Roumeguère. ×750.

3 median cells dark colored of which the upper 2 are fuliginous and opaque, the lower olivaceous, 14.4 × 8–9 μ; end cells hyaline, the apical cell short, conic, bearing a crest of 3 widely divergent setae, 16–25.6 μ, the basal cell short, obtuse, abruptly contracted into a short stipe, not exceeding 5 μ.

On living leaves of *Cliftonia ligustrina*, Ocean Springs, Mississippi, Nov. 1893, associated with ash or pale brown leaf spots, Herb. of F. S. Earle, Myc. Coll., U. S. Department of Agriculture.

10. PESTALOTIA SCIRPINA Ellis & Martin, Amer. Nat. **19**: 76. 1885.

Pustules lenticular to long hysteriform, scattered and distinct, subcuticular, later rupturing the cuticle longitudinally, contents at length oozing out and staining the matrix, 140–1000 × 110–140 μ.

Conidia fusiform, 5-celled, erect or usually curved, constricted at septa dividing the two lower colored cells, 20–24 × 6.5–8.5 μ; median cells dark colored, the upper two fuliginous, opaque, the lower olivaceous, 15–17 μ; apical hyaline cell conic, elongate, sometimes tapering, basal hyaline cell broad, conic, supported on an erect pedicel, 3.5–10 μ; crest mostly of 3 setae, sometimes 2, curved, divergent, 18–27 μ (Fig. 2, *2*).

On culms of *Scirpus maritimus,* Shores of Chesapeake Bay, Md., July, 1884, J. T. Rothrock in E. & E. No. Amer. Fung. *1626*; on dead culms of *Scirpus olneyi,* Point à la Hache, La., March, 1887, A. B. Langlois in E. & E. No. Amer. Fung. 2nd Ser., *2181.*

11. PESTALOTIA CURTA Sacc. Bul. Soc. Bot. Ital. **1904**; 209, fig. 5. 1904; Sacc. Syll. Fung. **18**: 480. 1906.

Acervuli usually concentrically disposed, largely epiphyllous, prominent, breaking through the epidermis in elongate fissures, 160–220 × 80–230 μ, issuing forth in dense black masses, irregularly scattered and sometimes confluent, seated on sub-circular, dark brown spots or areas of different shape usually marked by concentric zones above and lacking them below.

Conidia broad-clavate, 5-celled, usually curved and unequilateral, 20–24 μ long; median cells 14–16 × 8.5–10.5 μ, colored, the two upper umber or fuliginous, the lower olivaceous, constricted at the septum dividing the two lower cells; basal hyaline cell broad conic and small, superior cell narrow conoid or tapering and sometimes hidden, bearing 3 or rarely 2 or 4 setae which are sometimes branched, 16–30 μ; pedicel erect, 2–5 μ (Fig. 4, *11*).

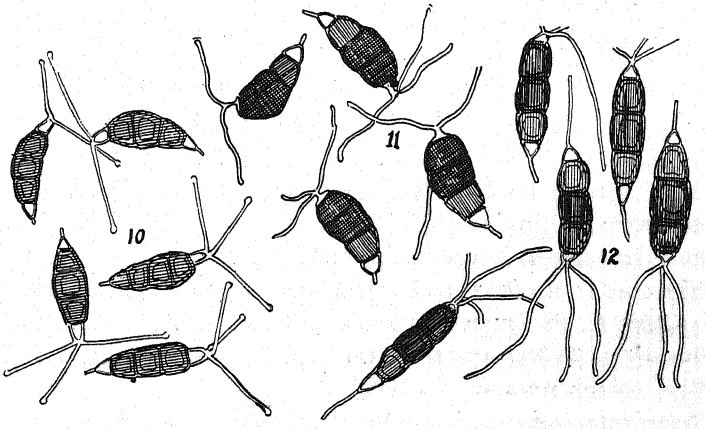


FIG. 4. Conidia: *10, P. palmarum* Cke. from Sydow Fung. Exot. Ex. *239,* on leaves of *Cocos nucifera,* Phillippines; *11, P. curta* Sacc. from D. Sacc. Myc. Ital. *1570,* on leaves of *Ceratonia siliqua; 12, P. vaccinii* (Shear) Guba from Myc. Coll., U. S. D. A., on leaves of *Vaccinium macrocarpon,* Parkdale, N. J. Sept., 1901, Shear, sub *P. guepini* Desm. var. *vaccinii* Shear. ×750.

On wilting leaves of *Ceratonia siliqua.* Associated with reddish or brown sub-circular spots or areas of different shape which sometimes coalesce.

Study is based on type specimens of *P. curta* Sacc. in D. Sacc. Myc. Ital. *1570,* Botanic Garden, Padua, on leaves of *Ceratonia siliqua,* April, 1904.

12. PESTALOTIA PALMARUM Cke. Grevillea **4**: 115. 1875, and **5**: 102. 1876. Pl. 86, fig. 3: Sacc. Syll. Fung. **3**: 796. 1884; Klebahn, Myc. Centralbl. **4**: 9. 1914.

P. phoenicis Vize, Grevillea **5**: 14. 1876; Sacc. Syll. Fung. **3**: 796. 1884; Klebahn, Myc. Centralbl. **4**: 11. 1914.

P. brevipes Prillieux and Delacroix, Bul. Soc. Myc. France **10**: 84–85. 1894; Sacc. Syll. Fung. **11**: 788. 1895; not Cke.

P. palmicola Sacc. and Syd. in Sacc. Syll. Fung. **14**: 1030. 1899.

Pustules black, sparse, sub-epidermal, later erumpent, amphigenous, 125–425 × 100–260 μ, circular or broad elliptic from above, sometimes coalescing, seated on spots or present generally on the dead areas.

Spores fusiform, straight or curved, 5-celled, 16.5–21 × 4.8–6.5 μ; interior cells olivaceous, the two upper occasionally darker, somewhat constricted at septa, 12–14 μ; exterior cells conical; setae 2 or 3, usually straight and spreading outward, 8.5–20 μ, often knobbed; pedicel short, 2–6.5 μ (Fig. 4, *10*).

On leaves of *Cocos nucifera* and other species of palm, associated with pale dead areas of various shapes surrounded by definite, reddish brown borders.

Specimens examined: On *Cocos nucifera* (sprout); Kew Herb., India, Jan. 27, 1870; (leaves) Baker, F. Malayana *65,* Los Banos, Philippines; Hume, Fungi of Florida *53,* Sanibel Island, Dec. 28, 1899; Roumeguere, Fung. Sel. Gall. Ex. *5166* and Thüm., Myc. U. *1676,* Demerara, British Guiana, M. C. Cooke, Herb. J. A. Stevenson *2220,* Rio Piedras, Porto Rico, 1914, Stevenson; Philippine Fungi *23,* Los Banos, O. H. Reinking; Sydow Fung. Exot. Ex. *289,* Taytay, Philippines, E. O. Merrill.

On *Howea foresteriana* (=Kentia foresteriana) and *H. belmoreana* (leaves); Office F. Pl. Quar. and Insp. U. S. D. A. *714,* Philadelphia, Pa., in greenhouse, J. T. Rogers; Myc. Coll. U. S. D. A., Florida, sub *P. discoidea* Ellis, nomen nudum.

On *Phoenix dactylifera* (leaves); Vize. Micro. Fung. Exot. *7,* Col. Hobson, sub *P. phoenicis* Vize; Kew Herb., East Indies, Col. Hobson, 1876, sub *P. phoenicis* Vize.

This species was described by Cooke, first on a dead sprout of germinating coconut from Bengal, India (spores 0.015 mm. long), and later on dead

leaves of the same host from Demerara, British Guiana (colored part of spores 0.045 mm. long). Both are *P. palmarum* but the dimensions given in each case are incorrect. The spores of the type on sprout of coconut from India lack the knobbed setae; otherwise the fungus is similar to the specimens later described from Demerara, British Guiana. Klebahn (43) considered *P. phoenicis* Vize a distinct species, but type material at Kew collected by Col. Hobson shows sufficient agreement with *P. palmarum* Cke. to regard Vize's name as a synonym. *P. brevipes* Prillieux and Delacroix is synonymous with *P. palmarum* Cke. Saccardo and Sydow (Sacc. Syll. Fung. **14**: 1030. 1899) renamed *P. brevipes* (Sacc. Syll. Fung. **11**: 788. 1895) to *P. palmicola* Sacc. and Syd. because there already existed a *P. brevipes* Cke. on *Aralia spinosa*. Both names, therefore, become synonyms. *P. discoidea* Ellis, on leaves of *Howea forestieriana*, is the same as *P. palmarum* Cke. The name and description of this fungus were never published, but specimens of the type accompanied by a description in Ellis' hand writing are deposited in Mycological Collections, United States Department of Agriculture.

Confusion has resulted from Cooke's descriptions of *P. palmarum*. The incorrect application of this name to other species of *Pestalotia* on a wide variety of plants has appeared extensively in mycological and pathological literature. Bernard (3, and 5, pp. 1–16) regarded the *Pestalotia* on tea and *P. palmarum* identical but it also appeared to Bernard that *P. guepini* and *P. palmarum* were distinct species and that the former was not the cause of the gray blight. Doyer (25) contended that *P. palmarum* and *P. guepini* were identical; but Sawada (69) regarded the tea fungus as a distinct species which he named *P. theae*; and Butler (13), who studied type material of these three species, confirmed their distinctiveness. Archer (1) confirmed the individuality of *P. palmarum* and *P. guepini* by their comparison in artificial culture; and Bertus (8, 9), by morphologic and cultural studies of *P. theae* and *P. palmarum*, reported these species distinct. My studies recognize the individuality of these species.

There has been much doubt concerning the biology of *P. palmarum*. Bernard (4) obtained infection on coconut palm by placing a cluster of diseased leaves in a healthy isolated tree; after two months, disease appeared. An uninoculated tree nearby remained healthy. He also found that a single conidium was capable of penetrating the leaf at several points, producing several spots close to one another. He reported that infection is stomatal, which fact has been confirmed by Stockdale (78). Stockdale found that the fungus was also capable of infecting the leaves through wounds and that it was frequently accompanied by *Diplodia epicocos*, a saprophyte which completes destruction begun by *P. palmarum*. Leefmans

(51) found that in Java the injury caused by the adults and larvae of the two-colored coconut leaf beetle, *Bronthispa froggatti,* was increased by *P. palmarum,* which penetrated the young leaves through injuries made by this insect. Bertus (9) found that *P. palmarum* is able to infect coconut leaves through wounds but not healthy tissue.

Doyer (25) failed to infect *Kentia foresteriana* with a form from *Cocos,* with a form from *Rhododendron* incorrectly named *P. guepini,* with Shear's strain of *P. guepini* Desm. var. *vaccinii* Shear [= P. vaccinii (Shear) Guba], and with a form from coconut incorrectly named *P. theae.* She concluded that the palm disease is of non-parasitic nature. Petch (60, p. 83; 61, pp. 226 and 262; and 62, pp. 163–164), Rutgers and Arens (66), and Rutgers and Dammerman (67) attributed the dry bark-girdle disease of rubber seedlings to *P. palmarum* Cke., but Steinmann (76) reported this disease due to sunburn.

The leaf spot of palms is prevalent in the tropics according to Morstatt (55, pp. 234–236), but its occurrence, according to Stockdale (78, 79) in the West Indies, Bernard (4, and 6, pp. 38–43) in Java, Knowles (44, pp. 26–27) in the Fiji Islands, and others is restricted to old or weak trees and unfavorable conditions for growth. Kowalski (45, pp. 56–58) reported that the disease occasioned considerable loss of young palms in the New Hebrides. Bernard (4) reported one-year-old coconut plants most susceptible in Java, and that the fungus caused great damage to coconut plantations. In the tropics in general this leaf disease is not of much concern and the primary factors responsible for damage have in most all cases been overlooked and wrongly attributed to *P. palmarum.* Prillieux and Delacroix (65) reported damage to species of *Kentia, Chamaerops,* and especially *Corypha australis* by the palm fungus in greenhouses at Versailles, France. Bernard (6, pp. 38–43) reported the fungus on *Oredoxa, Elais guinensis, Maniltoa gemmipara, Palaquium, Myrmecodia echinata, Thea,* and *Hevea brasiliensis.* A leaf disease of *Hevea* has also been attributed to *P. palmarum* by Bernard (7, pp. 43–44), Petch (60, 61, 62), Weir (88, p. 88), and others, but my studies indicate that the palm fungus is limited to members of the palm family.

13. PESTALOTIA THEAE Sawada Spec. Rept. Agr. Exp. Sta., Taiwan, Formosa, **11**: 113, pl. 4, figs. 7–9, T. 4, 1915 [Japanese; tr. Tanaka, Mycologia 9: 171, 1917].

Brownish spots coalescing and forming irregularly margined patches of variable extent especially common along leaf margins. Spots 1 cm. or more in diameter marked by concentric rings of alternate lighter and deeper shades.

Pustules punctate, chiefly epiphyllous, often arranged in concentric circles or scattered without order, 88–176 μ diam., sub-epidermal, on rupturing forming black crusts.

Spores long-fusiform, erect, 4-septate, slightly constricted at the septa, 23–30 × 7–8 μ; 3 middle cells 16.5–19 μ, dark olivaceous; end cells hyaline, 4–6 μ long, basal cell conic, apical cell cylindric with 2–4, commonly 3, hyaline, filiform appendages, 25–48 μ, the free ends usually knobbed; stalk filiform, rigid or curved to one side, 5–9 μ, sometimes knobbed (Fig. 3, *7*).

Fungus occurs on living leaves of tea causing disease known as gray blight. Large pustules appear on concentric zones but on the lower leaf surface the zones are not always distinct and the pustules are usually few. The fungus occurs generally in tea-growing regions.

Specimens examined: Sydow Fung. Exot. Ex. *146* Peradeniya, Ceylon, on leaves of *Camellia thea* (*Thea sinensis*) Nov. 1912, Petch, sub *P. palmarum* Cke.; Ceylon, May, 1927; Buitenzorg, Java, May, 1927.

Like *P. palmarum* the identity of the tea fungus was not known until Butler (13), Sawada (69), and Bertus (8, 9) recognized it as a distinct species, *P. theae* Sawada. Previously the fungus was incorrectly designated *P. guepini* Desm. and regarded of cosmopolitan host range.

P. theae attacks leaf blades, petioles, and stems of tea. The fungus grows from the blades through the petioles into perennial parts of the bush and causes cankers. Carruthers (14, pp. 4–7, and 15, pp. 5–6) and Petch (58, 59, and 63, pp. 27–30, 106–107, 191, 211) reported destruction of shoots following infection at the cut ends of stems left in gathering the tea, leaving a "crow's foot" structure of half a dozen dead twigs. Tunstall and Bose (86) successfully infected the cut shoots of tea with the gray-blight fungus. They reported that it frequently attacks margins of young immature leaves after a period of hot, dry weather causing rim blight, and that the attacks of red spider also make leaves susceptible to infection.

Carruthers (15) observed in Ceylon that gray blight appears after bushes are allowed to go 18 months without pruning. According to Tunstall (85, pp. 77–80) attacks of gray blight are an indication of unsatisfactory soil conditions. The fungus does not attack vigorous healthy leaves. Its attacks are confined to old damaged bushes or to those previously weakened by some other cause. Bertus (9) found that *P. theae* was able to infect tea and coconut leaves through wounds but not healthy leaves, while Doyer (25) failed to obtain infection of tea with a *Pestalotia* from *Cocos*, incorrectly designated *P. theae*, and with a *Pestalotia* from *Cocos* incorrectly designated *P. guepini*. Doyer regarded forms from *Cocos nucifera* and *Altingia* as *P. theae* Sawada because of the knobbed setae. She was apparently unaware of the fact that the setae of *P. palmarum* Cke. also have

knobbed extremities. Since *P. theae* is restricted to tea, inoculating tea with forms from palm, as Doyer has done, might be expected to give negative results. Speshnev (75) observed at Chakva, near Batum, that *Thea sinensis* was particularly susceptible and that the native species *Thea assamica* suffered much less damage. Other accounts of gray blight are made by Delacroix (22, p. 71), Delacroix and Maublanc (23, pp. 415–419), and Bernard (3, and 5, pp. 1–16).

14. PESTALOTIA MACROTRICHA Klebahn, Myc. Centralbl. **4**: 6. 1914.

Acervuli amphigenous, usually epiphyllous, subglobose or lenticular, black, punctiform, 140–280 μ wide, subepidermal, then erumpent, circular, surrounded by the torn epidermis, often densely gregarious and confluent, sooty on drying and spreading over the leaf surface.

Spores 25–31 × 7.4–9.4 μ, erect or sometimes curved, long fusoid, tapering to the base, 5-celled; 3 median cells olivaceous, long cylindric, equally colored or the upper two slightly darker than the lower, 18–23 μ, only slightly constricted at the septa; exterior cells prominent, the upper long cylindric with a crest of 3 or sometimes 2 or 4 coarse, long, divergent setae, 17–40 μ; the basal cell long conic with a long erect pedicel, 8–17 μ (Fig. 2, *3*).

On stems and leaves of *Rhododendron* and *Azalea* associated with definite dark or pale brown spots and areas commonly confined to the tips and along the margins of the leaves, confluent frequently and causing defoliation. The fungus appears on stems as black, raised pustules scattered without order.

Specimens examined: E. & E., Fung. Col. *370a* (type) on leaves of *Rhododendron maximum*, Nuttalburg, W. Va. Nuttal, sub *P. guepini* Desm.; on Rhododendron hybrids, Boskoop, Holland. Washington Insp. House, May 26, 1920, J. A. Stevenson; on *Rhododendron maximum*, Holland, Herb. J. A. Stevenson *1359*, Apr. 9, 1921; on *Rhododendron sp.* Westchester, Pa., 12–14–1914, F. Windle; on *Rhododendron ponticum*, Dedemsvaart, Holland, Washington Insp. House, 4–4–23, A. J. Bruman; brown fallen leaves of *Rhododendron* from Belgium, Washington Insp. House, 10–28–15, J. T. Rogers, Pl. D. S. *471*, sub *P. guepini* Desm.; on *Rhododendron sp.* Naarden, Holland, Washington Insp. House, Apr. 10, 1921, A. J. Bruman and H. F. Bain.

The long fusoid spores, olivaceous colored interior cells, the upper two of which at maturity are sometimes darker, long hyaline exterior cells, long setae and pedicel are characteristic of this species. The black raised fruiting pustules on leaves and stems are also typical of the fungus. Doyer's strains from *Taxus*, *Retinospora*, and *Biota* which she named *P. macrotricha*

Kleb. are not this fungus. No species occurring on conifers show any agreement with the one on *Rhododendron*.

The stem and leaf blight of *Rhododendron* or *Azalea* are commonly encountered in shipments of stock from Holland and France, and the disease causes considerable damage to rhododendrons, especially in nurseries. Clinton (18) reported the disease on imported rhododendrons in Connecticut, and Flachs (30) described the leaf blight on *Azalea* in Germany imported from Belgium. Schwarze (71, p. 112) reported *Pestalotia* on sunburnt leaves of *Rhododendron* in New Jersey which he named *P. guepini*, and I have found *Monochaetia* and other fungi on similarly affected foliage.

Schmitz (70) reported that the fungus is often found on leaves that have suffered from activities of a small aphid. By the usual methods of artificial inoculation he obtained positive evidence that this form is parasitic in nature. Tengwall (81, p. 58) also obtained infection. Doyer (25) inoculated rhododendron with a strain from *Cocos* incorrectly determined as *P. guepini* Desm., with Shear's *P. guepini* Desm. var. *vaccinii* Shear [=*P. vaccinii* (Shear) Guba] from cranberry, with strains from *Juniper* and *Rhododendron* named *P. funerea*, and a strain from *Retinospora* incorrectly named *P. macrotricha*. She obtained the rhododendron *Pestalotia* from the same plant (*R. ponticum*) on which Tengwall originally found his strain and with it inoculated the Pink Pearl rhododendron and *R. ponticum*. Leaves of rhododendron were also inoculated with two strains isolated from *Rhododendron* incorrectly named *P. guepini*, *P. funerea* from *Thuja*, a strain from *Taxus* incorrectly named *P. macrotricha*, and a strain from *Altingia* incorrectly named *P. theae*. Inoculations in every case were unsuccessful, and Doyer concluded that the form on rhododendron is not a parasite.

15. **Pestalotia rhododendri** (D. Sacc.) Guba, n. sp.
P. versicolor Speg. var. *rhododendri*, D. Sacc. Myc. Ital. *978*. 1902. nomen nudum.

Pustules black, epiphyllous, densely gregarious or scattered, often confluent, erumpent on maturity, tearing the epidermis irregularly, the black contents spreading over the matrix, 140–400 μ in diameter, seated without order on dried brown areas of living leaves.

Spores 5-celled, broad fusiform, erect or slightly curved, not constricted at septa, 21–27 μ; median cells stout and short, guttulate, the two upper fuliginous, the lower olivaceous, 16.5–18.7 × 8–10 μ; end cells hyaline, prominent, apical cell broad conic or short cylindric, basal cell broad conic, abruptly contracted into a short straight pedicel, 4–6.5 μ; setae 2 or usually 3, rarely branched, curved and widely divergent, 17–35 μ (Fig. 6, *22*).

On blighted or living leaves of *Rhododendron* associated with spots or dead areas usually along the margins and at the apex.

Specimens examined: D. Sacc. Myc. Ital. *978*, on leaves of *Rhododendron hybridum* and *R. ponticum*, Italy, sub *P. versicolor* Speg. var. *rhododendri* D. Sacc.; on blighted leaves of *Rhododendron obtusum* var. *amoenum*. Jamaica, Long Island, N. Y., Sept. 1924, P. E. Case.

This species differs from *P. macrotricha* Klebahn by the broader and shorter spores, the shorter setae, and the strongly contrasted color of the median cells.

16. PESTALOTIA GIBBOSA Harkness, Bul. Calif. Acad. Sci. **2**: 439–440. 1887.

Acervuli epiphyllous, sparse, rarely confluent, punctiform, erumpent, black, sub-globose, 70–140 μ wide, seated on pale, dead spots or dead areas with definite borders.

Conidia 5-celled, unequilateral, constricted at septum dividing the 2 lower colored cells, 25–30 μ; median colored cells 16–19 × 9–10.5 μ, the upper 2 fuliginous and subglobose, the lower olivaceous, sub-cylindric; end cells hyaline, the basal cell long conic, or somewhat tapering into a short, filiform pedicel up to 6.4 μ long, sometimes knobbed at the base; apical cell, long cylindric, bearing 2–4, usually 3, long, curved setae, 30–60 μ, coarse and widely divergent, often with capitate or knobbed extremities (Fig. 5, *16*).

On pale, dead spots and areas of living leaves of *Gaultheria shallon*, Seattle, Washington, Dec. 1893, C. V. Piper. The type of this species has not been available, but the original description of *P. gibbosa* agrees with the fungus studied. According to Harkness, the type collected at Point Reyes, California, June, 1886, and distributed in Pacific Coast F. *4130* frequently covers nearly the entire leaf, which is blackened with spores.

17. PESTALOTIA LEPROGENA Speg. Anal. Mus. Nac. Buenos Aires **23**: 119. 1912.

Spots repand-orbicular, somewhat large, separate, at length confluent, broadened irregularly, dark, leathery.

Acervuli loosely gregarious or scattered, black, conoid, erumpent, circinate, often confluent, sooty on maturity, 72–145 μ wide, blackening the surface in places.

Spores 5-celled, clavate, tapering to the base, curved or erect, 18.8–22.2 μ; median cells guttulate, colored, the 2 upper fuliginous, opaque, swollen, the lower olivaceous, 13–15.5 × 7.7–8.8 μ; end cells hyaline, large, broad conic, setae 3, slender, spreading, 15–26.6 μ, widely divergent; pedicel fragile, 4.5–7.7 μ (Fig. 6, *21*).

On mature fruits of *Musa sapientum* and *M. pedicellata* ex Brasilia, Buenos Aires and La Plata, Nov. 1911, Spegazzini.

Pustules are seated on spots which at first are 2–5 mm. in diameter, later larger, dirty in color and leathery, often covering the entire fruit, deforming and discoloring it. Stevens, Fungi of Porto Rico *122* sub *P. funerea* on leaves of *Musa paradisiaca,* differs from the type only in the length of the setae which measure 10–15.5 μ and in the lighter color of the upper two of the three median cells. The acervuli are largely epiphyllous, circinate, sooty at maturity, effuse, seated on large white spots or areas with dark brown or purple borders.

18. PESTALOTIA PALLIDICOLOR Speg. Ann. Mus. Nac. Buenos Aires III. **13**: 412. 1911; Sacc. Syll. Fung. **22**: 1227. 1913.

Acervuli epiphyllous, small, sparse, scattered, at first covered then erumpent, circinate, black, 72–125 μ wide, distinct.

Conidia erect, elliptic-fusoid, 16.5–20 μ, not, or only slightly, constricted at septa; median cells guttulate, pale olivaceous, equally colored, 12.2–13.3 × 5.5–6.6 μ; exterior hyaline cells short conic, basal obconic, apical cell mucroniform; setae 3, short, 8.8–15 μ; pedicel 2.2–6.6 μ, abruptly expanding into the basal cell (Fig. 5, *15*).

On dry or decaying scapes of *Synandrospadix vermitoxica,* Botanic Gardens, La Plata, Argentina, Oct. 1910, Spegazzini.

19. PESTALOTIA GRACILIS, Klebahn, Myc. Centralbl. **4**: 10–11. 1914. *P. sassafras* E. & E. nomen nudum, Ellis No. Amer. Fung. *1376.* 1885.

Fruiting bodies amphigenous, mostly hypophyllous, lenticular, subepidermal then erumpent, surrounded by the torn epidermis, 60–200 μ in diameter, densely gregarious and confluent, blackening the matrix, spores issuing in black cirrhi.

Spores 5-celled, erect, fusoid, sometimes slightly curved, slightly constricted at septa, 19.5–23 μ; lower median cell olivaceous, the upper 2 umber, 12–15 × 6–7 μ; basal hyaline cell long conic, tapering, apical cell large conic or cylindric bearing a crest of 2–4, usually 3, widely divergent setae, 13–25 μ; pedicel erect, 4–7 μ (Fig. 5, *14*).

On dead dried leaves of *Sassafras variifolia.* Sooty mats of spores cover both surfaces of the brown leaves. Study is based on type specimens in Ellis No. Amer. Fung. *1376,* on dead leaves of *Sassafras,* Newfield, N. J., Oct. 1884. Fungus is also distributed in E. & E., Fung. Col. *370b* on leaves of *Laurus sassafras* [= *Sassafras variifolium*], Newfield, N. J., Oct. 1883, sub *P. guepini* Desm.

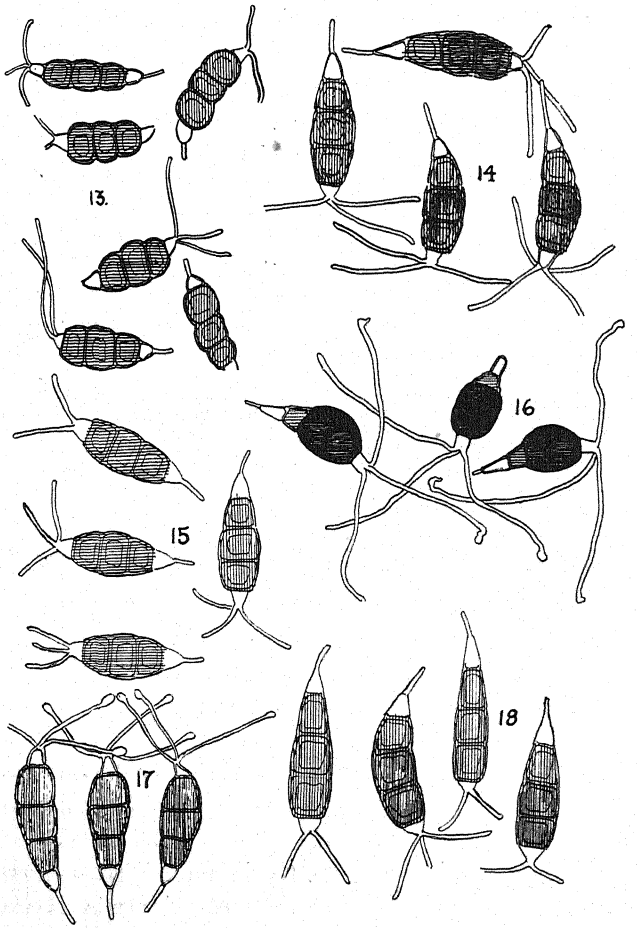


FIG. 5. Conidia: *13, P. torulosa* B. & C. from Herb. Berk. *5055,* at Kew, on seeds of *Citrullus vulgaris* So. Carolina; *14, P. gracilis* Klebahn from Ellis, No. Amer. Fung. *1376,* on leaves of *Sassafras,* sub *P. sassafras* E. & E.; *15, P. pallidicolor* Speg., on *Synandrospadix vermitoxica,* La Plata, Argentina, 1910, Spegazzini; *16, P. gibbosa* Harkness on *Gaultheria shallon,* Seattle, Washington, Dec., 1893, C. V. Piper; *17, P. clavata* Cke. & Ellis, No. Amer. Fung. *350,* on living leaves of *Smilax,* Sept., 1879; *18, P. copernica* Speg., on decaying fruits of *Copernica cerifera,* Puerto Leon, Argentina, 1909, Spegazzini. ×750.

Klebahn described *P. gracilis* on the basis of several different forms from unrelated hosts of which E. & E., Fung. Col. *370b* is the first listed. This specimen and the type of *P. sassafras* E. & E. are identical. A description of *P. sassafras* was never published; consequently the name is reduced to synonymy.

20. PESTALOTIA MENEZESIANA Bres. and Torr. Broteria (Bot.) **8**: 142. 1909; Sacc. Syll Fung. **22**: 1222. 1913.

Acervuli amphigenous, black, numerous or densely gregarious and coalescing freely, effuse and spreading over the surface, at first subepidermal, then erumpent, surrounded by the torn epidermis, 80–160 μ in diameter.

Conidia 5-celled, curved, broad at the top, tapering into a rather long conical basal cell, 16.8–21.6 μ; median cells differently colored, the 2 uppermost fuliginous, somewhat cask-shaped, the lower olivaceous, slightly constricted at the septa, 12–14.4 × 6.4–7.2 μ; basal cell long conic, apical cell narrow cylindric, often long; crest of 3 setae, 16–30 μ, divergent; pedicel short, erect, up to 5 μ (Fig. 3, *19*).

Occurring as a saprophyte on dried leaves of *Vitis vinifera.* Torrend, Fung. Sel. Ex. *185,* on leaves of *Vitis vinifera,* Porto Santo, Madeira Islands, A. de Noronha.

21. PESTALOTIA COPERNICA Speg. Anal. Mus. Nac. Buenos Aires, III. **13**: 411. 1911; Sacc. Syll. Fung. **22**: 1227. 1913.

Acervuli lenticular, conoid, 75–175 μ in diameter, circular, scattered or gregarious, freely confluent, forming black incrustations, subepidermal at first, then erumpent, generally distributed and darkening the matrix.

Conidia clavate, 5-celled, erect or sometimes slightly curved, tapering to the base, not, or hardly, constricted at the septa, 20–24.5 μ; median cells guttulate, olivaceous, the upper 2 sometimes slightly darker than the lower, 15.5–17.7 × 5.5–6.6 μ; end cells hyaline, the apical cell usually short conic bearing 2 or rarely 3 short, straight setae, 6–12 μ, divergent, basal cell hyaline, long, tapering into a short pedicel, 2.5–5 μ (Fig. 5, *18*).

On somewhat decayed, immature fruits of *Copernica cerifera,* existing as a saprophyte, Puerto Leon, Misiones, Argentina, June, 1909, Spegazzini.

22. PESTALOTIA CLAVATA Cke. & Ellis, Grevillea **6**: 85, pl. 99, fig. 7, 1878; Sacc. Syll. Fung. **3**: 796. 1884; Voglino, Sul. Gen. Pest. pl. 9, fig. 17. 1886.

P. grandis B. & C. in Crié, Ann. Sci. Nat. Bot. VI, **7**: 59, pl. 8, fig. 21, 1878, 1879. Voglino, Sul. Gen. Pest. p. 32; Sacc. Syll. Fung. **10**: 491. 1892. Curtis Geol. and Nat. Hist. Survey No. Car. Bot. III, 118. 1867. nomen nudum.

Acervuli numerous, crowded, 100–175 μ in diameter, amphigenous, sometimes entirely epiphyllous, lenticular, sub-epidermal then erumpent, seated on brown concentrically zoned spots with purple margins.

Spores fusiform, usually slightly curved, 5-celled, 25–31 μ, slightly constricted at septa; colored cells umber, sometimes olivaceous, equally colored,

16–21 × 7–8.5 μ; end cells hyaline, prominent, apical cell long cylindric, bearing 2–3 (usually 3) curved divergent setae; basal cell broad conic with a straight pedicel, 2.2–7.5 μ; setae 9–19 μ with a conspicuous knob at the extremities (Fig. 5, *17*).

On living leaves of *Smilax spp.* causing definite leaf spots. Specimens examined: Curtis Herb. *4046,* Alabama, from Peters *437,* sub *P. grandis* B. & C. on living leaves of *Smilax;* Ellis No. Amer. Fung. *350,* on living leaves of *Smilax.* Sept. 1879; Barth. Fungi Col. *4247,* on leaves of *Smilax rotundifolia,* Shreveport, La., Oct. 16, 1913, E. Bartholomew, associated with *Phyllosticta smilacis* E. & M.; Ellis, Fungi of New Jersey *2740,* on leaves of *Smilax,* Newfield, N. J., J. B. Ellis; Herb. S. M. Tracy *5242,* on leaves of *Smilax laurifolia,* Ocean Springs, Miss., S. M. Tracy, 5–2–1898, sub *P. funerea* Desm.; Herb. N. Y. State Museum, on living leaves of *Smilax glauca,* Babylon, Long Island, N. Y., Sept. 19, 1915, sub *P. guepini* Desm.

The original description of this fungus by Cooke and Ellis mentions 3-celled spores although the accompanying illustration shows 5 cells and 3 setae. Voglino (87) found 4-septate spores and setae with knobbed extremities in the type specimen.

This species was originally named *P. grandis* B. & C. by Curtis, but Berkeley, who described many of Curtis' specimens, failed to publish a description for it. Crié (20) cited this species and published an illustration of its spore which, however, offers an incorrect interpretation of *P. grandis.* Voglino listed *P. grandis* without its host or the source of the specimens. He furnished a brief description based on Crié's figure of the spore which, however, is untenable. Crié undoubtedly obtained type specimens from Berkeley, but since the description of *P. clavata* Cke. & Ellis antedates Crié's publication, *P. grandis* becomes a synonym.

23. PESTALOTIA STELLATA, B. & C., Grevillea **2**: 155. 1874. Sacc. Syll. Fung. **3**: 787. 1884.

Acervuli stellate, black, punctiform, sparse, amphigenous, flat, 140–280 μ in diameter, seated on definite pale, circular spots or irregularly shaped dead areas with black borders.

Spores 5-celled, erect or sometimes slightly curved, broad fusiform or broad elliptic, only slightly constricted at septa, 17.7–20 μ; 3 median cells cask-shaped, guttulate, 12–15.5 × 6.6–8.8 μ, the 2 upper colored umber, the lower olivaceous; end cells hyaline, small, short conic, the apical one bearing a crest of 3 broadly divergent setae, 4.4–11 μ; pedicel attenuated downwards (Fig. 6, *20*).

Pale definite spots on living leaves of *Ilex opaca,* usually secondary and associated with other fungi. Specimens examined: Herb. Curtis *4921* at Harvard, Society Hill, So. Carolina, Apr. 1855, and similar specimens in

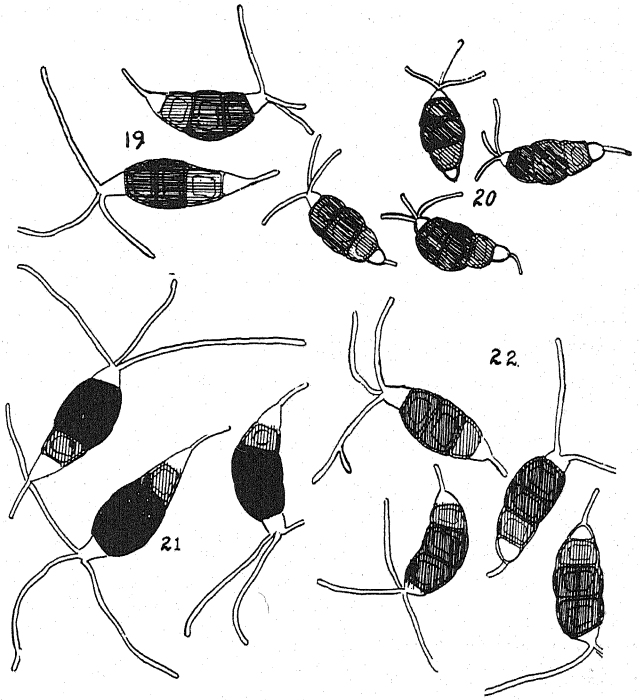


FIG. 6. Conidia: *19, P. menezesiana* Bres. & Torr., on *Vitis vinifera,* Madeira Islands; *20, P. stellata* B. & C. from Herb. Berk. *4921,* at Kew, on *Ilex opaca,* So. Carolina; *21, P. leprogena* Speg. on *Musa sp.,* La Plata, Argentina, Spegazzini; *22, P. rhododendri* (Sacc.) Guba from D. Sacc. Myc. Ital. *978,* on leaves of *Rhododendron hybridum* and *R. ponticum,* sub *P. versicolor* Speg. var. *rhododendri* D. Sacc. ×750.

Berkeley's herbarium at Kew; Myc. Coll. U. S. D. A., Liberty, Texas, Nov. 6, 1914, H. Jolivette sub *P. funerea.*

Cooke and Ellis (Grevillea **6**: 85. 1878) reported this species on *Quercus tinctoria* in Ellis, Fungi of New Jersey *2640,* and Saccardo (Syll. Fung. **3**: 787. 1884) subsequently cited both hosts. The form on *Quercus* lacks the pale white spots and contrary to the opinion of Cooke and Ellis is distinct from *P. stellata* B. & C. The fungus in Ellis No. Amer. Fung. *1352* labelled *P. stellata* on leaves of *Quercus tinctoria* and *Vaccinium arboreum* is not this species. According to Voglino (87), specimens of *P. stellata* in Ravenel, Fung. Amer. Ex. *254* on leaves of *Nerium* and in E. & E., No. Amer. Fung. *34* on leaves of *Ilex opaca* belong to this species. Both are different species, the latter is *P. annulata* B. & C. and the former a new species.

24. PESTALOTIA TORULOSA, B. & C. Grevillea **2**: 155. 1874; Sacc. Syll. Fung. **3**: 795. 1884.

Acervuli minute, scattered and frequently confluent, sub-epidermal, then exposed, flat or usually round, numerous, black, punctiform, 72–190 μ in diameter, usually seated on black spots.

Spores 5-celled, clavate, torulose, or strongly constricted at the septa, usually curved, 17.5–20 μ; the colored cells olivaceous, guttulate, 13.3–15.5 × 5.5–6.6 μ; end cells hyaline, the apical one cylindric, the basal cell long conic tapering into a short pedicel about 2.2 μ; setae 2–3 divergent 6.6–11 μ (Fig. 5, *13*).

On seeds of watermelon, *Citrullus vulgaris*, So. Carolina in Curtis Herb. *5035* at Harvard and similar specimens in Berkeley's herbarium at Kew.

25. PESTALOTIA VIRGATULA Klebahn, Myc. Centralbl. **4**: 13. 1914. *P. funerea* Desm. *f mangiferae* Sacc. Atti Istit. Veneto Sci. VI, **2**: 461. 1884.

Pustules small, black, scattered, largely hypophyllous.

Conidia 5-celled, clavate, 18–22 × 6.5–7.8 μ, the two upper colored cells umber or fuliginous, lower olivaceous; setae 3 or rarely 2, relatively stout, 18–21 μ, widely divergent.

On dead leaves of *Mangifera indica* in herbarium of A. Brunaud, specimen of which carries the name "*Sphaerella tahitensis* in association with *P. funerea*. On leaves of Manguier, Tahiti, A. Brunaud."

The fungus shows no relation to *P. funerea* Desm. Klebahn evidently was unaware of a published description of this fungus. His erection of the new name and the segregation of the fungus from *P. funerea* Desm. was correct.

26. PESTALOTIA VERSICOLOR Speg., Michelia **1**: 479. 1879; Sacc. Syll. Fung. **3**: 790. 1884.

Pustules sparse, globose-lenticular, at first covered then erumpent, raised, surrounded by shreds of the torn epidermis and cuticle, 80–215 μ wide, on maturity discoloring the surrounding matrix.

Conidia 5-celled, clavate, erect or somewhat curved, only slightly constricted, 22–26.5 μ; median cells colored, guttulate, the two uppermost cask-shaped, fuliginous, the lower olivaceous, 15–17.7 × 7.8–8.8 μ; end cells hyaline, prominent, the apical one usually short cylindric and bearing 3, rarely 4, coarse widely divergent setae, 17.7–26.5 μ; basal cell long and broad conic often inflated, descending into a short straight pedicel, 4–7 μ (Fig. 7, *24*).

On fallen decaying leaves of *Nerium oleander,* Conegliano, Italy, 1876, Spegazzini.

The entire matrix is colored pale gray and the black pustules are distributed without order. Other fungi are present. Klebahn (43) included all 5-celled spore forms with median cells of contrasted color in *P. versicolor.* In these forms considered, the upper two median cells are dark in contrast to the lower olivaceous colored cell. The opaque cells are cask-shaped or sub-globose. Within the species *P. versicolor,* Klebahn has included many forms from different hosts, based upon Saccardo's compilation of this species and his study of certain exsiccati. Type specimens were not considered. Specimens in D. Sacc. Myc. Ital. *978* named *P. versicolor* var. *rhododendri* on leaves of *Rhododendron hybridum* and *R. ponticum* and in Barth. Fung. Col. *2441* named *P. guepini* on *Ribes rubrum* are considered by Klebahn to belong to *P. versicolor.* These forms according to my studies of the types do not belong to this species.

Klebahn (42, 43, 14–16) successfully infected a wide range of plants with a form from *Darlingtonia* which he determined to be *P. versicolor.* From this fact he assumed that the fungus was of world-wide distribution. His knowledge of the fungus was based on the original description which lacks many details necessary to a good understanding of the species. His form on *Darlingtonia* has spores measuring 26–31 μ while those of the type measure 22–26.5 μ. Klebahn's conception of the type is incorrect and his form on *Darlingtonia* belongs elsewhere.

Klebahn was able to infect *Darlingtonia* under bell-jars but not in open greenhouses. He asserted that this form was essentially a saprophyte capable of existing under moist conditions on different substrata but not causing infection of living or freshly wounded tissue.

27. PESTALOTIA MOLLERIANA Thümen, Myc. Univ. *1988,* 1881.

Pustules amphigenous elevated, usually scattered and solitary, circular, densely gregarious and confluent, at first subepidermal later erumpent, freed, blackening the matrix, tearing the epidermis irregularly, 140–280 μ wide, without spots.

Conidia fusiform, narrowed at the ends, usually erect, equilateral, 5-celled, only slightly constricted at septa, 19–22 × 6.3 μ; median cells guttulate, olivaceous, equally colored, 12.7–14.9 μ; end cells hyaline, basal one broad conic, abruptly contracted into a short stalk, 4–7 μ, apical cell conic or cylindric with a crest of 2–3 setae, 10–16 μ, widely divergent, curved (Fig. 7, *23*).

On fallen dried leaves of *Eucalyptus sp.* Oct. 1879, Choupal, Portugal, Möller in Thümen, Myc. Univ. *1988* and Roumeguère Fung. Sel. Gall. Ex. *5167.* Thümen stated that the spores are 5-septate. My study shows that they have 5 cells.

28. PESTALOTIA AQUATICA E. & E., Jour. Myc. 5: 157. 1889; Sacc. Syll. Fung. 10: 492. 1892.

Spots amphigenous, chestnut brown, definite, irregular in shape and size, as much as 3 cm. long and 2 cm. wide, concentrically wrinkled, surrounded by a purplish or dark border. Acervuli epiphyllous, sparse, scattered, erumpent, circinate, black, 80–130 μ in diameter, raised, convex then concave.

Conidia 5-celled, obovate, erect or slightly curved, not constricted at the septa, 20–24 μ; median cells guttulate, olivaceous, the upper 2 somewhat darker, the lower lighter in contrast, 13–15.6 × 7.2–8.4 μ; end cells hyaline, short conic, the apical one with a crest of 3 stout setae 10–20 μ, unequal in length and widely divergent; pedicel erect, 2.4–4 μ (Fig. 7, *26*).

Occurring as a saprophyte on living leaves of *Peltandra virginica*, Newfield, N. J., Aug. 27, 1889, Myc. Coll., U. S. D. A.

29. PESTALOTIA VERMIFORMIS Massee, Kew Bul. Misc. Inform. 1901: 156. 1901; Sacc. Syll. Fung. 18: 479. 1906.

Acervuli amphigenous, largely hypophyllous, solitary and scattered or crowded and confluent, subepidermal then erumpent, black contents issuing in cirrhi, present over entire leaf surface, 60–210 μ in diameter.

Spores 5-celled, erect often slightly curved, hardly constricted, clavate, tapering toward the base, 17.5–20 × 5.5–6.2 μ; 3 median cells olivaceous, equally colored, guttulate, 11–13.7 μ; end cells hyaline, the apical one cylindric or conic with 2–3, usually 3, simple setae, broadly divergent, 11–16 μ, basal cell long conic, tapering, pedicel erect, 2.2–5 μ (Fig. 7, *25*).

On living leaves of *Eugenia sp.*, Mt. Cook, Endeavour River, Queensland, Bailey, in Kew herbarium bearing the number *1132*. The pustules are present generally over the leaf surface. Massee stated that the pustules are epiphyllous and that the spores are 4–5 septate. Examination of type material shows that they are largely hypophyllous and that the pustules are not confined to pallid spots. The spores are not 25–32 μ long as the type description indicates and the septa are uniformly four. Spores with four median colored cells were not observed.

30. PESTALOTIA INQUINANS Cke. & Harkness, Grevillea 12: 94. 1884; Sacc. Syll. Fung. 3: 784. 1884.

Acervuli amphigenous, largely epiphyllous, numerous, 140–280 μ in diameter, subepidermal then erumpent, surrounded by the torn epidermis, coalescing freely and forming large spore cushions, the contents on maturity spreading over the matrix, leaving sooty deposits.

Spores 5-celled, erect, clavate-fusoid, slightly constricted at the septa, 22.2–26.6 μ; median cells guttulate, dark olivaceous, equally colored, 15.5–

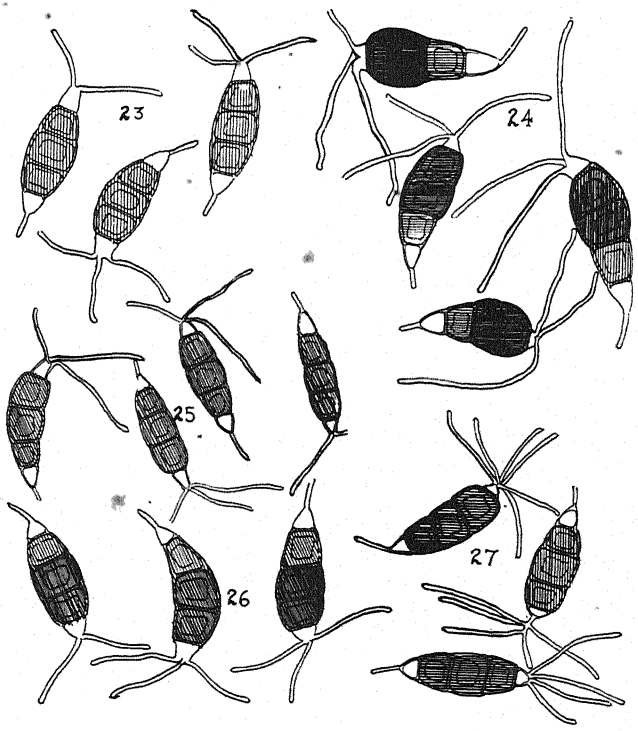


FIG. 7. Conidia: *23, P. molleriana* Thüm., from Thüm. Myc. Univ. *1988,* on leaves of *Eucalyptus sp.; 24, P. versicolor* Speg. on *Nerium oleander,* Conegliano, Italy, 1876, Spegazzini; *25, P. vermiformis* Massee, from Kew *1132,* on *Eugenia sp.,* Mt. Cook, Endeavour River, Queensland, Bailey; *26, P. aquatica* E. & E., from Myc. Coll., U. S. D. A., on *Peltandra virginica,* Newfield, N. J., 1889; *27, P. inquinans* Cke. & Hark. from Kew *2094,* on *Eucalyptus,* California. ×750.

17 × 7.5–8.8 μ; end cells hyaline, the apical one conic or short cylindric bearing a crest of 4–6, usually 4 or 5, curved setae, 11–20 μ, extending forward or sidewards; basal cell large conic with a short pedicel, 4.4–8.8 μ (Fig. 7, *27*).

On fallen dead leaves of *Eucalyptus sp.,* California, in Kew herbarium bearing the number *2094.*

The pustules are present on both leaf surfaces, densely gregarious in places, forming sooty deposits. The presence of numerous setae are characteristic of the spores of this species. The fungus exists in association with other fungi.

KEY TO SPECIES INCLUDED IN PART I

The usefulness of this key is limited to those species of *Pestalotia* listed in the species index. In using this key, mean dimensions and typical characters should be emphasized. Aberrances should not be considered.

Conidia 5-septate; fruiting body an acervulus seated on a stroma, usually pezizoid	*P. pezizoides* de Notaris
Conidia 4-septate; fruiting bodies either acervuli or pycnidia, stroma lacking	
Colored cells 10–16 μ long	
Conidia 4.8–6.6 μ wide	
Setae 1–4, frequently branched	*P. guepini* Desm.
Setae 3, sometimes 2, rarely branched	
Setae 6–11 μ long, conidia torulose	*P. torulosa* B. & C.
Setae 9–20 μ long, conidia not torulose	
Setae knobbed at extremities	*P. palmarum* Cke.
Setae not knobbed	
Colored cells very pale	*P. pallidicolor* Speg.
Colored cells olivaceous; pustules circular	
Conidia elliptic-fusoid, about 6.5 μ wide	*P. molleriana* Thüm.
Conidia narrow-fusoid, 5.5–6.2 μ wide	*P. vermiformis* Massee
Colored cells umber, pustules elongate	*P. foedans* Sacc. & Ellis
Upper two colored cells umber, pustules circular	*P. gracilis* Klebahn
Setae 20–30 μ long	*P. vaccinii* (Shear) Guba
Conidia, 6.4–10.5 μ wide	
Setae 4–11 μ long	*P. stellata* B. & C.
Setae 10–30 μ long	
Apical cell long, cylindrical or conic; basal cell long, conic or tapering	
Upper two colored cells umber, lower olivaceous	*P. gracilis* Klebahn
Upper two colored cells fuliginous	
Conidia 6.4–7.2 μ wide	*P. menezesiana* Bres. & Torr.
Conidia 7.7–8.8 μ wide	*P. leprogena* Speg.
Apical cell short, cylindric or conic; basal short, obtuse	
Colored cells olivaceous or upper two slightly darker than lower	*P. aquatica* E. & E.
Upper two colored cells fuliginous	
Pustules elongate, concentrically arranged	*P. curta* Sacc.
Pustules circular, punctiform	
Conidia obovate	*P. cliftoniae* Tracy & Earle
Conidia clavate	*P. virgatula* Klebahn
Colored cells 15–18 μ long	
Conidia 5.5–6.6 μ wide	*P. copernica* Speg.
Conidia 6.5–9.5 μ wide	
Setae 5–20 μ long	
Colored cells olivaceous, conidia elliptic-fusoid	*P. inquinans* Cke. & Hark.
Colored cells umber, conidia clavate	*P. funerea* Desm.
Upper two colored cells fuliginous, conidia obclavate	*P. glandicola* (Cast.) Guba
Setae 18–27 μ long, not knobbed; colored cells fuliginous	
Pustules hysteriform	*P. scirpina* Ellis & Martin
Pustules circular	
Conidia 7.8–8.8 μ wide	*P. versicolor* Speg.
Conidia 8.5–9.5 μ wide	*P. sphaerelloides* Ellis & Langl.
Setae 25–48 μ long, knobbed; colored cells olivaceous	*P. theae* Sawada
Colored cells 16–23 μ long	
Conidia 7–10.5 μ wide	
Setae 7–19 μ long	
Setae usually 3, knobbed	*P. clavata* Cke. & Ellis
Setae usually 5, frequently knobbed	*P. cesatii* Rabenhorst

Setae 17–60 μ long	
Colored cells olivaceous, equally colored or upper two slightly darker	
Setae knobbed	*P. theae* Sawada
Setae not knobbed	*P. macrotricha* Klebahn
Upper two colored cells fuliginous	
Conidia elliptic-fusoid	*P. rhododendri* (Sacc.) Guba
Conidia obclavate	*P. gibbosa* Hark.

SPECIES INDEX TO PART I

Good species are in roman type, synonyms are in italics, and new species are in bold-face roman type.

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P. clavata Cke. & Ellis 22
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P. compacta B. & C. 1
P. conigena Lév. 5
P. copernica Speg. 21
P. curta Sacc. 11
P. foedans Sacc. & Ellis 6
P. funerea Desm. 5
var. *mangiferae* Sacc. 25
P. gibbosa Harkness 16
P. glandicola (Cast.) Guba 7
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P. grandis B. & C. 22
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P. pallidicolor Speg. 18
P. palmarum Cke. 12
P. palmicola Sacc. & Syd. 12
P. pezizoides de Notaris 1
P. phoenicis Vize 12
P. ramosa D'Almeida 1
P. rhododendri (Sacc.) Guba 15
P. sassafras E. & E. 19
P. scirpina Ellis & Martin 10
P. sphaerelloides Ellis & Langl. 8
P. stellata B. & C. 23
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P. torulosa B & C. 24
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P. vermiformis Massee 29
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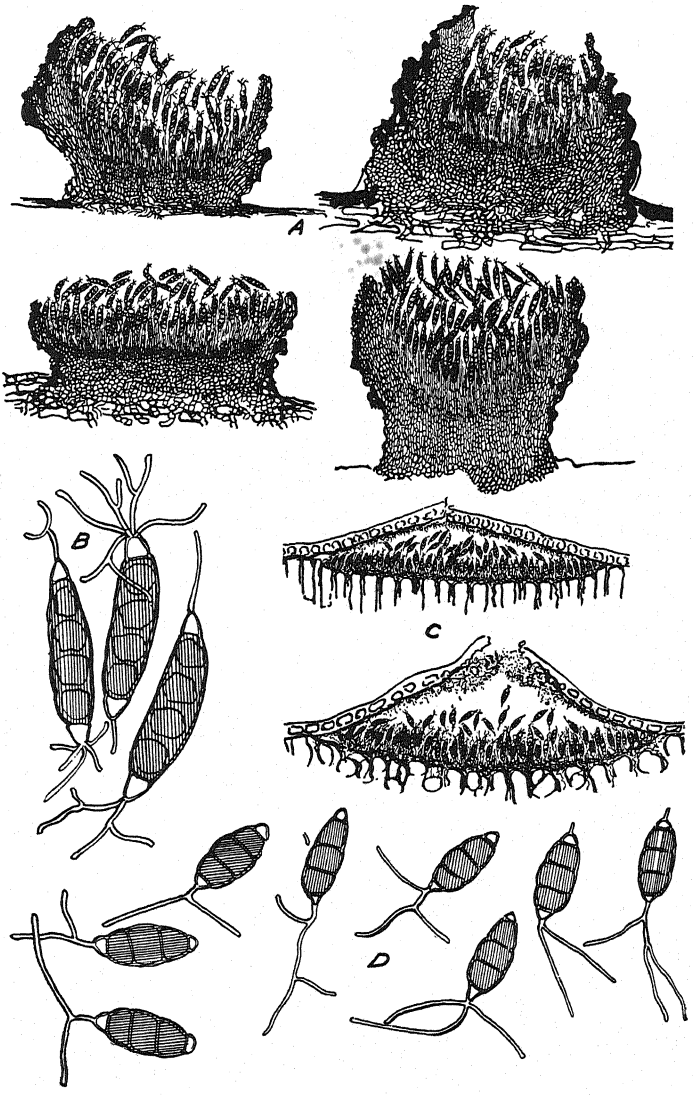
EXPLANATION OF PLATE I

A. Section through fruiting pustule of *P. pezizoides* de Notaris from Ellis, No. Amer. Fung. *35*, on canes of *Vitis vinifera.*

B. Conidia of *P. pezizoides* de Notaris from Michener Fungi **19**: 82, *2790.* Myc. Coll., U. S. D. A., on canes of *Vitis sp.* not *Humulus lupulus,* sub *P. compacta* B. & C. ×750.

C. Sections through fruiting pustules of *P. guepini* Desm. from Myc. Coll., U. S. D. A., on leaf of *Camellia japonica* in greenhouse, Washington, D. C., May, 1907, W. A. Orton, sub *P. camelliae* Pass.

D. Conidia of *P. guepini* Desm. from Desm. Pl. Crypt. Fr. Fasc. 22, *1084,* on leaves of *Camellia japonica.* ×750.


A
B
C
D

THE EFFECT OF THE H-ION CONCENTRATION OF THE CULTURE SOLUTION ON THE BEHAVIOR OF FUSARIUM CROMYOPHTHORON AND ALLIUM CEPA AND THE DEVELOPMENT OF PINK-ROOT DISEASE SYMPTOMS[1]

C. P. SIDERIS

INTRODUCTION

The pink-root disease of onions is of great importance to the onion growers of California. Official reports (40) estimate field losses at 14 per cent without taking into consideration, at the same time, storage losses which have resulted from the same trouble. The disease is not confined to California, but has a very wide distribution, having been reported from every onion-growing section in the United States, Canada, Australia, and the Bermuda Islands.

All reports on the pink-root disease point out definitely that the causal parasite is an organism belonging to the genus *Fusarium*. Taubenhaus and Mally (37), of the Texas Agricultural Experiment Station, who have made extensive studies on the occurrence of the pink-root disease in Texas, have determined and also named the pathogenic organism as *Fusarium malli* Taub. This contribution as a whole is a compilation of careful observations and experimental results concerned with the factors influencing the development of this disease under field conditions.

THE DISEASE IN CALIFORNIA

The pink-root disease occurs in more than one locality in California. The writer has often received onions with pink-root symptoms sent by farm advisors from various parts of the state.

One of the largest onion-growing sections of California is the Delta district of Stockton. This district is composed mainly of a number of small islands in the Sacramento river. The soil consists almost entirely of peat derived from a vegetation that flourished in the past, representatives of which may still be found in certain uncultivated marshy areas. The underground system of irrigation is practiced by the majority of growers, this proving to be very inexpensive and extremely satisfactory.

In spite of the uniform conditions that one finds in fields of this type, with respect to soil texture, organic matter, and distribution of water there are areas, in any given field, in which the chemical properties differ greatly,

[1] Thesis submitted to the Graduate Division of the University of California in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

although the physical properties of the soil may be relatively uniform. Such differences may be found more often in the acidity and salt concentration of the aqueous extract of the soil. The differences in the acidity of different soil samples may run as high as 2.0 pH values. It was particularly noticeable in certain fields that had not been leveled perfectly that the pH value of the aqueous extracts of the soil taken from the higher spot was greater than that taken from the lower spot. Such differences in pH value may be attributed to differences in the concentration of salts resulting from differences in the rate of evaporation of water.

The physicochemical differences of the aforesaid localized field areas were reflected in the growth and condition of the plants grown thereon. The plants on the high-leveled areas were slow growing and unhealthy, whereas those on the low-leveled areas were very luxuriant and healthy. This striking coincidence of soil conditions with poor plant development led the writer to suspect that soil conditions might be partly, if not wholly, responsible for the weakened and unhealthy condition of the plants. The factors that may be responsible for the development of this condition are: (a) soil acidity, (b) relative salt concentration, and (c) rapid changes in the moisture content of the two different field areas. Other causes believed to operate concurrently with the above are parasitic or facultative microorganisms. Examinations of the roots of many such plants proved that the most frequently occurring microorganisms were species of the genus *Fusarium*.

PATHOLOGICAL AND CULTURAL TECHNIQUE

Isolation of Microorganisms from Root Tissues

The technique employed for the isolation of the various microorganisms associated with tissues of diseased roots was as follows. The root or roots were first thoroughly washed with ordinary tap water, then transferred to sterilized tap water, with which they were washed as much as possible until all soil particles had been removed, and then placed between sheets of sterilized filter paper for dehydration. At the end of this operation the root, or roots, selected for examination were put in a sterile petri dish where they were sectioned by sterile instruments into very small pieces. Each piece was transferred into a petri dish containing nutrient agar. The nutrient agar used for this purpose was prepared in three distinct lots, each one with a different degree of acidity. Lot A had a reaction of pH 4.0; B, 6.8; and C, 7.5. The main reason for adopting such a procedure was to encourage or discourage the growth of various micoorganisms. This method of isolation yielded many microorganisms, the majority of which were ordinary saprophytes of the soil. Beside these, some species of

Fusarium, a *Rhizoctonia,* a *Phoma,* a *Cephalosporium* and a number of species of *Penicillium* and *Aspergillus* were obtained. Fusarium organisms were, however, invariably associated with diseased tissues; for which reason their possible relationship to pink-root disease was studied. A certain species of *Fusarium,* occurring quite often in diseased tissues in between 30 and 40 per cent of the cases, was selected for these studies.

REVIEW OF PREVIOUS WORK

The pink-root disease of onions is a comparatively new disease, and the literature associated with it is relatively meager. The first report of its occurrence in the United States was made in 1913, but no studies were undertaken until 1917. The first real contribution is that of Taubenhaus and Mally (37).

A number of species of *Fusarium* have been studied in the past by various investigators in connection with many root-rot and wilt diseases on a variety of plants. Nearly all these studies are concerned more with moisture and temperature, as factors contributing to the development of root-rot or wilt diseases, than with the relative acidity of the soil solution. There are a number of excellent researches on the physiological behavior of Fusarium spp. in a number of culture media at different H-ion concentrations. Although some of these researches give a very good idea of the behavior of Fusarium spp. when in contact with a number of organic substances, most of them have produced no information on the parasitic potentiality of the organisms when in contact with living tissues of different hosts.

The studies of Young and Bennett (46) on *Fusarium oxysporum* Scht. in culture media at different H-ion concentrations give the behavior of this fungus as follows: ". . . the reaction of the culture solution in which *F. oxysporum* was grown continued to become acid until an H-ion concentration of pH 3.6 was reached, then turned toward alkalinity, and growth continued until all the organic compounds were broken up and an H-ion concentration of pH 8.4 was reached." MacInnes (24), in a similar study on a wheat scab *Fusarium,* found that ". . . as growth proceeds, the H-ion concentration of the solutions is changed by the organism, those on the acid side tending to become more alkaline and those on the alkaline side more acid." Johnson (16), in studying seven soil molds, including *Fusarium bullatum* and *oxysporum,* found that ". . . the optimum reaction for the molds lies more to the acid side of the neutral point than toward the alkaline, practically all of them making nearly a maximum growth as low as pH 2.0, while they are cut off quite sharply at pH 8.7. . . . The reaction of all the solutions was changed toward a more alkaline value except the

neutral solution with *F. bullatum*. . . . In no case did the molds reach the pH values which were found to inhibit their growth in the studies on the effect of hydroxyl ions.'' Webb (42), in studying the germination of spores of a species of *Fusarium*, at different H-ion concentrations, found that ''. . . the range of the spore germination with respect to the reaction of the medium is between pH 2.0 and pH 10.0. Maxima generally appear between pH 3.0 to pH 4.0 and near pH 7.0.'' Karrer (19), in determining the action of amylase secreted by a species of *Fusarium* at different H-ion concentrations, found that ''. . . the maximum total accumulation was produced in the solution having an initial pH of 4.5 and a final pH of 7.8.'' Hopkins (15), in his studies on *Gibberella saubinetii* Sacc. in its relation to the wheat-scab disease, found that ''. . . the fungus has two maxima, one on the acid and the other on the alkaline side. The maximum rate of infection coincides with the maximum growth of the organism.'' Johnson (17) reports as follows on the effect of acidity on the Fusarium wilt of tobacco: ''In one experiment all plants died at the three highest soil acidities, one died in each of the next three lower reactions and none died in the three jars at the alkaline end, although finally they all became infected. In another experiment all plants died in the first five grades of reaction from the acid.'' He furthermore states that . . . ''the evidence seems fairly conclusive that an acid soil favors the wilt disease, although it may occur in neutral or alkaline soils.'' Rast (27) observed in fertilizer experiments that ''. . . cotton wilt may be controlled with the use of fertilizers containing potash.'' Taubenhaus and Mally (37) state that *F. malli* may produce pink root disease in soils of both acid and alkaline reaction.

The behavior of Fusarium spp. in causing rot or wilt on a variety of plants at different temperatures has been studied by many investigators with excellent results. Link (20) found that the optimum temperature for the growth of *F. oxysporum* was 30° C., while that for *F. trichothecioides* Wr. was 12° C. Goss (7), using the same organism in studies on rot production, found that *F. oxysporum* grew more rapidly at 25° C. than *F. trichothecioides* and produced a more extensive rot on the potato tuber, the latter being able to produce a rot at much lower temperatures. Jones and Tisdale (18), in their studies on the influence of soil temperature on the development of flax wilt, found that ''The minimum or critical soil temperature for the disease is about 14° C., with 38° C. as the maximum and the optimum for extreme disease development about 24° to 28° C.'' Tisdale (39), in similar studies, found that optimum temperature for growth and infective power of *F. lini* was 26°–28° C., with minimum 11° and maximum 34° C. Clayton (4), in his studies on the relation of soil temperature to the development of the Fusarium wilt of tomatoes, found that the

optimum temperature for the growth of the fungus was 28° C., and for the development of the disease 22°–32° C. Mann and Nagpurkar (21), in their studies on the Fusarium blights of potatoes in India, found that ". . . with an increase of temperature wilting of plants increases as well as rotting of tubers in storage." Dickson (5), in studying the influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by *Gibberella saubinetii* found that ". . . the parasite functions normally over a comparatively wide range of temperatures, *viz.*, 3° to 32° C. The optimum temperature for spore germination, vegetative growth and sporulation is about 24° C. on unacidified and 28° C. on acidified media. The most favorable soil temperature for the blighting of wheat ranges from 12° to 28° C., while that for corn is from 8° to 20° C."

Moisture, as a factor influencing the production of rot in potatoes by species of *Fusarium,* has been studied by Goss (7), who found that ". . . the relative humidity plays a very important rôle in determining the progress of tuber rots, and has the same influence on all three species *viz.*, *F. oxysporum, F. radicicola,* and *F. trichothecioides.* With a high humidity at a given temperature the rotting was always greater than at a temptera-ture 5° to 10° higher but with a low humidity." Wilcox, Link, and Pool (44) obtained a greater percentage of infection in cultures kept in saturated air than in others over Ca Cl_2.

The literature in connection with the physiological behavior of the onion plant is very meager. Rogers (29) states that onion plants require large amounts of moisture and moderate temperatures from the time of planting until they are mature.

In summarizing the results of the above workers one arrives at the following conclusions: (a) That species of *Fusarium* in culture media are able to increase or decrease the initial acidity or alkalinity of these media; (b) That the optimum temperature for the growth of these organisms and infection of their hosts lies at 20° to 30° C.; and (c) That species of *Fusarium* grow fairly well in moist media, and infection increases with increments of moisture plus temperature to a certain degree.

FUSARIUM SPECIES ASSOCIATED WITH ONION ROOTS

The number of Fusarium species that one may find associated with either healthy, dying, or dead roots is not definite. These organisms, being soil inhabitants, may be found on onions grown in acid as well as alkaline soil, in cold as well as in warm climate, and in moist as well as dry regions. The writer (32), in a survey of the Fusarium species associated with onion roots of plants grown in the Delta district, near Stockton, California, found that nearly 20 different species and varieties could be isolated from injured

or dead roots. Another *Fusarium* isolated from onion roots by Hanzawa (8) and Walker and Tims (41) is *F. cepae* Hanz. Hanzawa's organism, according to private information from Dr. Sherbakoff, is probably similar to that described by the writer (32) as *F. cromyophthoron* Sid. This organism, however, according to Hanzawa's description, either has not been investigated in connection with the development of pink-root symptoms or does not produce these symptoms, and, according to Walker's and Tim's statement, causes a dry to semi-watery decay of the scales with yellowing and premature dying of the tops. The prevailing opinion, therefore, as to whether *F. cepae* is identical with *F. cromyophthoron* is not definite.

The studies reported herein were confined to *F. cromyophthoron,* because of its constant association with onion roots showing pink root symptoms. A study of other species of *Fusarium* found on diseased tissues is being omitted at this time because of their less frequent association with the disease symptoms.

The name *F. cromyophthoron* is maintained throughout this presentation. This does not mean, however, that this organism is not closely related to, or identical with, *F. cepae.*

PHYSIOLOGICAL BEHAVIOR OF FUSARIUM CROMYOPHTHORON

Cultural Methods

Pure cultures of *F. cromyophthoron* were grown in dextrose solution and onion decoction in which a constant pH value was maintained with occasional introductions of adjusting reagents, such as 0. 2 N HCL and KOH.

Inorganic salts were used for the preparation of a 2 per cent dextrose solution in the following proportions.

Distilled water	1000 cc.
$MgSo_4$	2.12 grams
$Ca(NO_3)^2$	0.71 do
KH_2PO_4	1.36 do
$Fe(NO_3)_3$, 5% solution	1 cc.

An onion decoction also was prepared by cutting 200 gms. of peeled onions into small pieces and boiling them in 500 cc. of water that had been acidified previously with 25 cc. of 5N HCL. The mixture was boiled for half an hour, then filtered through cotton and the filtrate treated with 15 cc. of 5N NaOH.

Portions of 500 cc. of these solutions were placed in 1-liter Erlenmeyer flasks and adjusted to different hydrogen-ion concentrations before their

TABLE 1.—*Volume (cc.) of reagent (0.2N KOH) required per liter to adjust H-ion concentrations between pH values of 4.0 and 8.0*

pH	4.0	4.5	5.0	5.2	5.4	5.6	5.8	6.0	6.2	6.4	6.6	6.8	7.0	7.2	7.4	7.6	7.8	8.0
4.0	.....	0.4	0.8	1.0	1.8	2.6	5.0	6.0	7.6	9.2	11.5	14.5	17.5	21.7	25.9	30.8	33.7	36.5
4.5	.....	.....	0.4	0.6	1.4	2.2	4.6	5.6	7.2	8.8	11.1	14.1	17.1	21.3	25.5	30.4	33.3	36.1
5.0	.....	.....	.....	0.3	1.0	1.8	4.2	5.2	6.8	8.4	10.7	13.7	16.7	21.0	25.1	30.0	32.9	35.7
5.2	.....	.....	.....	.....	0.8	1.6	4.0	5.0	6.6	6.2	10.5	13.5	16.5	20.7	24.9	29.8	32.7	35.5
5.4	.....	.....	.....	.....	.....	0.8	3.2	4.2	5.8	7.4	9.2	12.7	15.7	20.0	24.1	29.0	31.9	34.7
5.6	.....	.....	.....	.....	.....	.....	2.4	3.4	5.0	6.6	8.9	11.9	14.9	19.1	23.3	28.2	31.1	33.9
5.8	.....	.....	.....	.....	.....	.....	.....	1.0	2.6	4.2	6.5	9.5	12.5	16.7	20.9	26.8	29.7	32.5
6.0	.....	.....	.....	.....	.....	.....	.....	.....	1.6	3.2	5.5	8.5	11.5	15.7	19.9	25.8	28.7	31.5
6.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	1.6	3.9	6.9	9.9	14.1	18.3	24.2	27.1	29.9
6.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2.3	5.3	8.3	12.5	16.7	22.6	25.5	28.3
6.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.0	6.0	10.2	14.4	20.3	23.2	26.0
6.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	3.0	7.2	11.4	18.3	21.2	24.0
7.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.8	8.4	16.3	19.2	22.0
7.2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.2	12.1	15.0	17.8
7.4	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	7.9	10.8	13.6
7.6	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.5	9.0
7.8	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	6.5
8.0	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

inoculation with *F. cromyophthoron*. Solutions of 0.2N KOH and H_2SO_4 were used for adjusting the reaction of the culture media. Table 1 was prepared to facilitate the work. The volume of KOH indicated as necessary for shifting the reaction of the medium from a higher to a lower acidity was determined on sterile dextrose media composed as mentioned above. With inoculated media all the values have to be changed slightly to overcome the interference of the various colloidal substances released in the solution by the organism. This interference may be approximately determined after brief experience with the organism.

The apparatus described by the writer in another paper (31) was used for the growth of the organism under sterile conditions and at adjusted hydrogen-ion concentrations. The adjusting reagent was added to the culture medium after a determination had been made of its hydrogen-ion concentration. Such adjustments of the pH value were made daily, this having proved very satisfactory and practical. The volume of the culture medium withdrawn from the flask for a single determination of pH averaged between 5 and 10 cc.

Changes in pH Produced by Fusarium Cromyophthoron on Media of Different Initial pH

The organism was grown in both types of media mentioned previously at different hydrogen-ion concentrations. The changes produced in these media as the result of the metabolic activities of the organism were recorded as closely as possible. Tables 2 and 3 contain the information on the utili-

TABLE 2.—*Changes in the pH of dextrose culture solution produced by Fusarium cromyophthoron together with tests indicating presence or absence of free sugars.* (See figure 1.)

Date, 1923	Changes produced by *Fusarium cromyophthoron* in cultures with different initial pH											
	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar
Apr. 10...	3.0	+	4.0	+	5.0	+	6.0	+	7.0	+	8.0	+
13...	3.2	+	4.0	+	5.3	+	5.8	+	6.8	+	7.8	+
15...	4.0	+	4.2	+	5.2	+	5.3	+	6.4	+	7.4	+
18...	3.6	+	3.7	+	5.0	+	5.1	+	6.2	+	7.2	+
20...	3.6	+	3.7	+	4.5	+	4.0	+	6.0	+	6.2	+
23...	3.8	+	3.8	+	4.0	+	3.8	+	5.3	+	5.9	+
25...	3.8	+	3.8	+	3.8	+	4.1	+	5.0	+	5.6	+
30...	3.8	+	4.6	−	3.8	+	5.2	−	4.8	+	5.6	+
May 4...	4.2	+	4.8	−	4.0	+	5.6	−	4.6	+	5.5	+
9...	4.6	−	5.2	−	4.3	−	5.8	−	.....	−	.....	−

TABLE 3.—*Changes in pH of onion decoction medium produced by Fusarium cromyophthoron together with tests indicating presence or absence of free sugars*

Date, 1923	Changes produced in the initial pH by the fungus											
	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar	pH	Sugar
Apr. 10...	3.8	+	4.2	+	4.6	+	5.7	+	6.0	+	7.0	+
13...	4.0	+	4.6	+	4.6	+	5.5	+	6.0	+	7.0	+
16...	3.9	+	4.2	+	5.0	+	5.5	+	6.0	+	7.0	+
18...	4.0	+	4.2	+	5.0	+	5.5	+	6.0	+	6.8	+
20...	4.1	+	4.3	+	5.0	+	5.3	+	5.8	+	6.8	+
23...	4.2	−	4.4	+	5.0	+	5.3	+	5.8	+	6.4	+
25...	4.2	−	4.5	−	5.2	−	5.4	−	5.6	+	6.0	+

zation of the sugar content of the solution by the organism and also changes in pH values. Figure 1 presents the changes in pH graphically. Tables 4 and 5 and figure 2 indicate the volume of each reagent required in the different cases to maintain constant pH values.

TABLE 4.—*Volume of adjusting reagents, in cc., required to neutralize the changes produced in the initial H-ion concentration of dextrose solutions of different pH by Fusarium cromyophthoron*

Date, 1923	pH determinations and volumes of reagent required for adjusting pH											
	pH	HCL	pH	KOH	pH	KOH	pH	KOH	pH	KOH	pH	KOH
Apr. 10	3.0	0	4.0	0	5.0	0	6.0	0	7.0	0	8.0	0
13	3.2	1	3.8	1	4.8	2	5.8	3	6.8	2	7.8	4
15	3.1	1	4.0	1	4.7	4	5.8	5	7.0	5	7.7	9
18	3.1	1	4.0	1	4.8	5	5.8	7	6.8	7	7.7	16
20	3.0	1	3.8	2	4.8	6	5.9	8	6.7	9	7.6	18
23	3.1	1	3.8	2	4.8	7	5.8	8	6.8	10	7.6	20
25	3.0	1	4.0	1	4.9	3	6.0	6	6.7	10	7.6	20
27	3.0	2	4.0	1	4.9	1	6.0	4	6.7	10	7.7	20
30	3.0	2	4.0	0	5.4	0	6.0	0	6.8	12	7.8	25
May 4	3.2	2	4.3	0	5.8	0	6.0	0	6.8	12	7.7	25
9	3.2	2	4.6	0	5.8	0	6.0	0	6.8	12	7.7	20
11	3.2	2	4.7	0	6.0	0	6.0	0	6.8	10	7.8	15
29	5.6	0	6.7	0	7.2	0	7.4	0	7.4	0	8.4	0
Total		16		9		28		41		99		192

The results obtained indicate that *F. cromyophthoron* is capable of changing the initial reaction of its substratum by the liberation of either acidic or basic substances, this depending on the initial reaction of the solution. The magnitude of the produced changes may be measured by

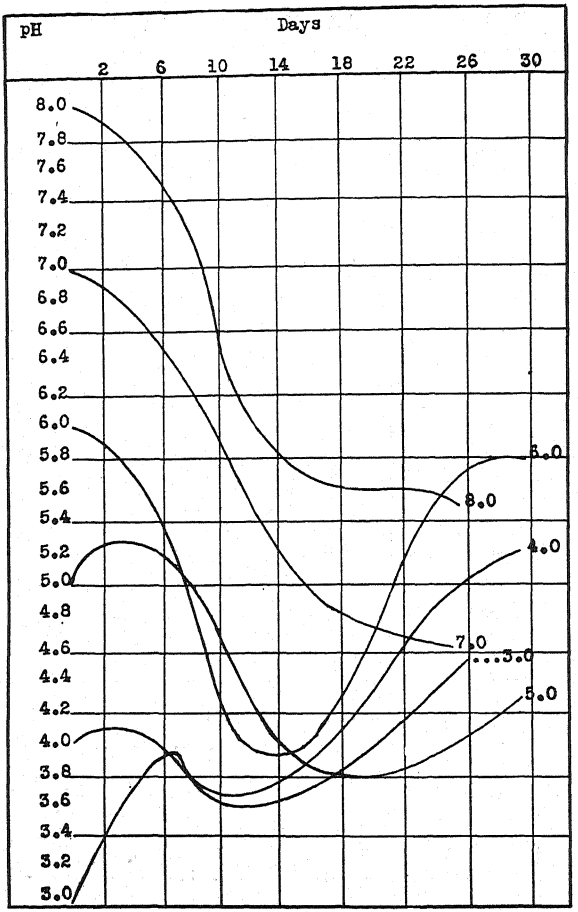


FIG. 1. Changes produced in the pH value of the various dextrose culture solutions by *F. cromyophthoron*. (See table 2.)

TABLE 5.—*Volume of adjusting reagents, in cc., required to neutralize the changes produced in the initial pH of onion decoction by Fusarium cromyophthoron*

Date, 1923	pH determinations and volumes of reagents required for adjusting pH											
	pH	HCL	pH	KOH	pH	KOH	pH	KOH	pH	KOH	pH	KOH
Apr. 10	3.0	0	4.0	0	5.0	0	6.0	0	7.0	0	8.0	0
13	3.0	1	4.3	1	5.1	1	6.0	2	6.8	3	7.7	15
16	3.2	2	4.0	1	5.0	0	5.8	3	6.8	6	7.8	25
18	3.1	2	4.1	2	5.0	0	5.8	4	6.8	8	7.7	30
20	3.0	2	4.0	2	5.0	0	6.0	5	6.7	10	7.8	30
23	3.1	3	4.2	2	5.0	0	5.7	4	6.7	13	7.7	35
25	3.0	3	4.0	1	5.0	0	5.8	4	6.8	15	7.7	35
30	3.0	2	4.0	0	5.0	0	6.0	0	7.0	0	7.9	30
May 30	5.8	0	6.2	0	6.8	0	7.4	0	7.6	0	8.6	0
Total		16		9		1		22		55		200

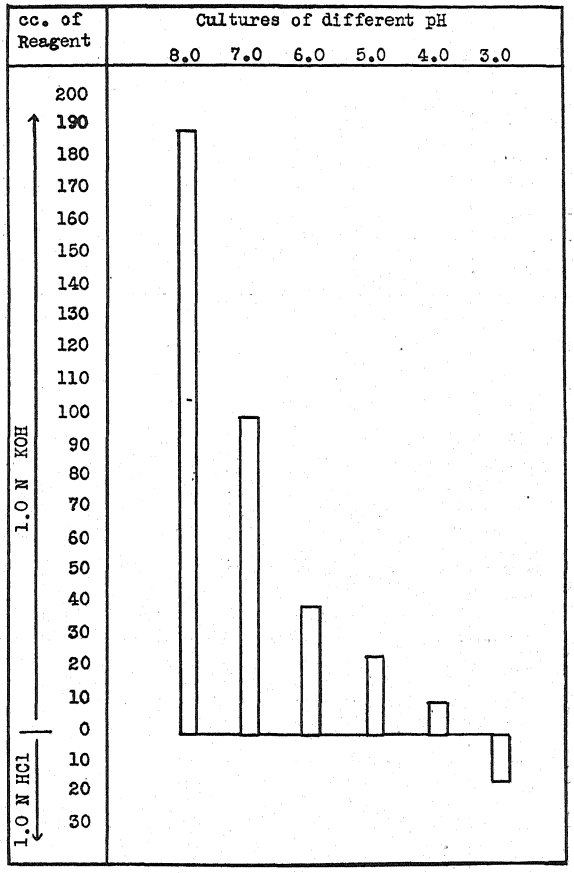


FIG. 2. Volume of 0.2 N KOH and HCl required to maintain the initial pH of dextrose solutions inoculated with *F. cromyophthoron*.

the volume of the reagent used in every case. Tables 2 and 3 indicate that the formation of acids may cease and alkalinity may develop in media after all the sugar has been consumed by the organism.

Vegetative Growth of Fusarium Cromyophthoron at Different pH Values

A number of 1-liter Erlenmeyer flasks equipped with the necessary accessories for controlling pH under aseptic conditions (31) were used for the growth of the fungus. To each flask 500 cc. of the dextrose solution were added, and the pH values were adjusted in each case previous to inoculation with the fungus. Four different series were employed labeled A, B, C, and D, and representing growth periods of 7, 15, 22, and 29 days. All inoculations were made the same day, using as nearly as possible, the

same amount of inoculum. The amount of mycelium produced at the end of the growth period of each series was recovered from the flask by filtration through a hardened filter paper of known weight. Both mycelium and paper were dehydrated in every case and then weighed. The results are recorded in table 6.

TABLE 6.—*Grams of dried mycelium produced in onion decoction at adjusted H-ion concentrations of pH 3.0, 4.0, 5.0, 6.0, and 7.5 by 7-, 15-, 22- and 29-day-old cultures of Fusarium cromyophthoron*

Series	Age in days	Weight in grams of mycelium in cultures of different pH					
		3.0	4.0	5.0	6.0	7.0	7.5
A	7	0.073	0.175	0.230	0.240	0.170	0.150
B	15	0.193	0.454	0.478	0.504	0.500	0.485
C	22	0.188	0.302	0.387	0.400	0.252	0.355
D	29	0.278	0.288	0.307	0.380	0.325	0.327
Total		0.732	1.219	1.402	1.524	1.247	1.317

The organism grew best at pH 6.0 during the 15-day growth period. This indicates that the arrested development of the organism beyond the fifteenth day is due to the depleted supply of sugars in the culture media and also to autolysis. Autolysis did not occur at pH 3.0, where also no decrease in the growth of the organism had been observed throughout all four series. Fehling's-solution tests proved that the organism had utilized completely the sugars in the cultures of pH 4.0, 5.0, 6.0, and 7.5 of series C and D, but not in that of pH 3.0. The low pH value of the last culture may account for the slower utilization of sugars and inhibition of autolysis. It seems that autolysis is inevitable after the organism has used up the total food substances of the culture media. The rise of the pH value of the solution during autolysis is due to the formation of ammonia resulting from the breaking down of the protoplasmic proteins of the dead fungus hyphae.

The Effect of Temperature on Vegetative Growth

The dextrose solution mentioned was used with 2 per cent agar for the preparation of a solid medium. The mixture was adjusted to different H-ion concentrations before sterilization. Duplicate samples were used to determine any changes in the pH value after sterilization. For the growth studies of this fungus petri-dish cultures were found very suitable. About 15 cc. of the medium were poured in a petri dish and after solidification a few spores of the fungus from a sporodochium were planted in the center. Five petri dishes were used for each pH value and each temperature. The

growth of the organism was determined by measuring the diameter of the colony at intervals of 48, 70, 96, 120, and 144 hours. The results obtained from these measurements are recorded in table 7.

TABLE 7.—*Diameter growth in mm. of colonies of Fusarium cromyophthoron on dextrose agar of different H-ion concentrations at 20° and 28° C.*

Age of culture, hours	Temperature, degrees C.	Growth in mm. of colonies in cultures of different initial pH				
		4.0	5.0	6.0	7.0	8.0
48	20	9	10	10	10	9
70	20	19	20	20	21	17
96	20	28	28	28	30	25
120	20	38	39	40	41	37
144	20	50	51	51	53	50
70	28	34	34	33	32	32
96	28	41	44	46	43	41
120	28	57	59	61	60	58
144	28	70	74	75	76	75

The results indicate that temperature alone, independent of the H-ion concentration of the culture medium, influences appreciably the vegetative growth of the organism. Higher temperatures, such as 28° C., favor a much more rapid growth than lower temperatures. The influence of the H-ion concentration of the culture medium on the growth of the organism was rather negligible at both higher and lower temperatures. The ineffectiveness of H-ion concentration in solid media may be attributed to the extremely slow movement of such ions to places where a decrease of their concentration has taken place by ions of opposite electric charge.

Discussion of Results

The results obtained on the behavior of *F. cromyophthoron* in culture media of different H-ion concentrations indicate that different concentrations of H-ions influence the metabolic processes and development of the organism. Temperature may, in an analogous manner, stimulate or retard the development of this organism. The changes produced in the pH reaction of the culture solution may be attributed to the formation of acidic or basic metabolic by-products by the fungus. Whether such acids are released by the organism or formed in the solution as the result of sugar assimilation is not known. If the latter is true it is to be assumed that the sugar molecule is oxidized into acids by the enzymes of the organism before assimilation. Such a process, if possible, will contribute to an accumulation of

acids, and in turn to an increase in the acidity of the culture solution. Acids may not be the only substances formed during metabolism. Basic substances may also be formed by analogous processes. The results presented in table 2 at pH 3.0 and in table 3 at pH 3.0 and 4.0 support this contention. The writer (36) has observed that acidic substances may be formed during metabolic activities at pH values above that of the isometabolic point of the substance concerned, and basic substances may be formed below this point. Weimer and Harter's (43) findings on *F. acuminatum,* Wolf's (45) on *Bacillus carotovorus,* Harter and Weimer's (9 and 10) on *Rhizopus tritici,* and Paine and Chaudhuri's (25) on *Bacillus solanisaprus* prove beyond any doubt that organisms are more apt to produce acid reaction during the assimilation of carbohydrates, and basic reaction when proteins are utilized. The writer (36) has found that if peptone alone is used in media there is no acidic reaction.

Young and Bennett (46), MacInnes (24), Karrer (19), and Hopkins (15) have found that alkalinity may occasionally be produced by various organisms. Such an alkalinity, in the cases cited, may be due to ammonia resulting from the hydrolysis of peptone, or protein of the media, or from autolysis.

In regard to the influence of temperature on the development of various species of *Fusarium,* different investigators have found that not only the development of these organisms is influenced by temperature, but their pathogenicity also. Link (20), Clayton (4), Goss (7), Jones and Tisdale (18), and Tisdale (39) have found those temperatures which favor a rapid development of Fusaria favor, in a similar manner, the development of rots and wilts. Bisby (2) has found that Fusaria grow best at temperatures between 20° and 25° C. As different species, however, vary considerably in their thermal response, Bisby's statement does not apply throughout the entire group of species of the genus *Fusarium.*

PHYSIOLOGICAL BEHAVIOR OF ALLIUM CEPA

The following physiological studies were conducted for the purpose of obtaining some information on the behavior of the normal onion plant, this being essential for comparative studies under pathological conditions.

CULTURAL METHODS

Onion bulblets and seeds were germinated on sand and allowed to grow for 15 days, until they had produced a growth of 10–20 cms. in both tops and roots, and then were transferred to stoneware jars of approximately 8 liters capacity. The seedlings were suspended, in the case of water cultures, through a 12 × 12 × 1-inch wooden board into which had been bored 10 holes, ¾ inch in diameter. Only one seedling was inserted in each hole, making the total number in each crock 10.

The culture solution used for the growth of the seedlings consisted of 2.12 gms. Mg SO_4, 0.71 gms. Ca $(NO_3)_2$, 1.33 gms. KH_2PO_4 and 1 cc. of a 5 per cent $Fe(NO_3)_3$ solution per liter. Different portions of this solution were adjusted to different H-ion concentrations by the addition of 0.2 N, H_2SO_4, and KOH. The soil cultures were prepared by mixing equal volumes of Oakley sand and peat. Different volumes of 3 N of H_2SO_4 or KOH were added to produce the desired H-ion concentration in the different soil cultures.

BEHAVIOR OF ALLIUM CEPA AT DIFFERENT H-ION CONCENTRATIONS

The behavior of onion plants at different H-ion concentrations was carefully observed and recorded. The effect of the pH on the plants was studied in two different ways: In the first case the changes that had been produced in the H-ion concentration of the culture solution by the growth of the plants were not adjusted, but in the second all such changes were adjusted by the addition of various volumes of 1 N H_2SO_4 or KOH. Determinations of the pH of the different solutions were made at rather frequent intervals, and any changes that had resulted in the second case were properly attended to. The results obtained by growing plants in cultures of different pH are recorded in tables 8 and 9.

TABLE 8.—*Changes in pH produced by Allium cepa growing in culture solutions with different initial H-ion concentrations*

Date, 1922	Changes in pH of the ten different cultures									
	1	2	3	4	5	6	7	8	9	10
May 22	4.0	5.3	5.3	5.5	5.5	6.0	6.0	6.5	6.5	8.0
25	4.2	5.4	5.4	5.6	5.5	6.0	6.0	6.4	6.4	—
29	4.3	5.5	5.7	6.0	5.6	6.0	6.0	6.4	6.4	—
31	4.4	5.7	5.9	6.0	5.8	6.0	6.0	6.5	6.6	—
June 1	4.8	5.8	5.9	6.0	5.8	6.2	6.2	6.5	6.7	—
4	5.1	6.0	6.0	6.0	6.0	6.2	6.3	6.6	6.8	—
6	5.5	6.0	6.0	6.3	6.0	6.4	6.0	6.7	6.7	—
9	5.4	6.0	6.0	6.5	6.0	6.2	6.0	6.7	6.6	8.0
15	5.4	6.0	6.0	6.6	6.0	6.2	6.6	6.6	6.3	7.5
19	5.2	6.0	6.0	6.7	6.0	6.5	6.6	6.8	6.8	7.5
22	5.3	6.0	6.0	6.7	6.2	6.6	6.6	6.8	6.8	7.4
24	5.3	6.0	6.0	6.7	6.3	6.6	6.6	6.9	6.8	7.4
27	5.3	6.0	6.0	6.7	6.5	6.6	6.6	6.8	6.8	7.3
30	5.4	6.0	6.0	6.7	6.5	6.5	6.6	6.7	6.7	7.3
July 1	5.3	6.0	6.0	6.7	6.5	6.5	6.5	6.6	6.5	7.2
4	5.3	6.0	6.0	6.6	6.4	6.5	6.6	6.6	6.6	7.0

TABLE 9.—*Volume, in cc., of normal HCL or KOH required to neutralize the changes produced in the pH of the culture solution by Allium cepa, and readings of the prevailing atmospheric temperature during day and night*

Date, 1923	Temperature, degrees F.		pH of cultures and volume of reagents used to adjust pH																	
	Day	Night	pH	HCL	pH	HCL	pH	HCL	pH	HCL	pH	HCL	pH	reagent	pH	KOH	pH	KOH	pH	KOH
Jan. 4	68	55	4.0		4.5		5.0		5.5		6.0		6.5		7.0		7.5		8.0	
9	72	52	4.0		4.5		5.0		5.5		6.0		6.5		7.0	8.0	7.3	10.00	8.0	
15	70	50	4.0		4.5		5.0		5.5		6.2		6.5		7.1		7.5		7.7	20.0
22	60	50	4.4	0.5	5.0	0.5	5.1		5.5		6.2		6.5		7.0		7.5		8.0	
25	80	45	4.3	0.5	4.8	0.5	5.3	0.5	5.5		6.1	0.7	6.5		7.2	2.0	7.5		7.9	
27	60	45	4.4	1.0	5.0	1.0	5.2	0.5	5.5	0.5	6.1	0.3	6.5		7.2	5.0	7.4	5.0	7.8	10.0
30	75	45	4.2	1.0	4.7	0.5	5.0	1.0	5.5	0.5	6.1	0.5	6.5		6.8		7.5		8.0	
Feb. 2	70	45	4.2	2.0	4.7	0.5	5.0	0.5	5.5		6.1	0.5	6.5		7.0		7.5		8.0	
6	70	45	4.2	3.0	5.0	2.0	5.2	1.5	5.5	0.5	6.1	0.3	6.5		7.0		7.3	5.0	7.8	10.0
9	82	48	4.2	2.0	4.5		5.0		5.6	1.0	6.1	0.2	6.5		7.0		7.5		8.0	
13	85	50	4.0	1.0	5.0	2.0	5.0	1.0	5.6	1.0	6.0		6.5		7.0		7.4	5.0	7.7	10.0
15	90	55	4.5	5.0	4.7	1.0	5.2	1.5	5.5	0.2	6.1	0.5	6.5		7.0	1.0	7.5	1.0	7.9	5.0
16	88	52	4.0		4.3		5.0	0.5	5.5	0.3	6.0		6.5		7.0	1.0	7.5	1.0	8.1	
19	80	55	4.0		4.8	2.0	5.0	1.0	5.5	0.5	6.0		6.5		7.0	1.0	7.4	4.0	8.0	
22	80	55	4.0		4.5		5.0		5.5		6.0		6.5		7.0		7.5			
24	85	55	4.5	4.0	4.7	2.0	5.2	1.5	5.7	2.0	6.0		6.5		6.9	2.0	7.3	5.0	7.6	15.0
Mar. 2	75	50	4.0		4.5		5.0		5.5		6.0		6.5		7.0		7.4	4.0	7.8	10.0
Total reagent				20.0		12.0		9.5		6.5		3.0		None		20.0		40.0		80.0

The results obtained, on the behavior of onion seedlings in culture solutions of different hydrogen-ion concentrations, indicate that the plants increased the pH in certain cultures and decreased it in others.

The changes were more pronounced at the two extremes, namely, at pH 4.0 and 8.0, and less in the cultures of intermediate pH reactions. The magnitude of the changes produced may be better understood by comparing the total volumes of the reagent used for adjusting the reaction of the solution (Table 9 and Fig. 3). There were no changes at pH 6.5 or, if any, they were very slight and difficult to detect. The changes in the cultures of higher or lower pH value were, however, quite appreciable.

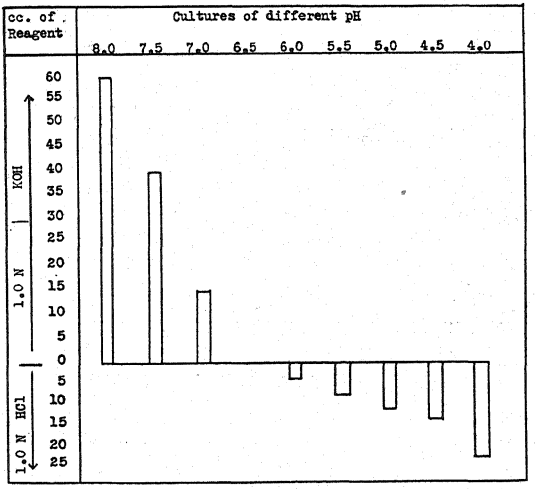


FIG. 3. Volume of 1.0 N KOH and HCl required to maintain the initial pH value of the various culture solutions in which *Allium cepa* was growing.

TABLE 10.—*Dry weights in grams of lots of 10 plants of Allium cepa growing in culture solutions*

Series	Date, 1922	Weight in grams of plants growing in culture solutions of different pH							
		4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5
A	May 22 to July 4	20.0	21.0	28.5	29.8	25.0	20.0	14.0	5.0
B[a]	Jan. 4 to Mar. 2	13.0	16.0	26.0	29.0	28.5	34.5	30.0	18.0

[a] Series B consists of the cultures reported on in table 9.

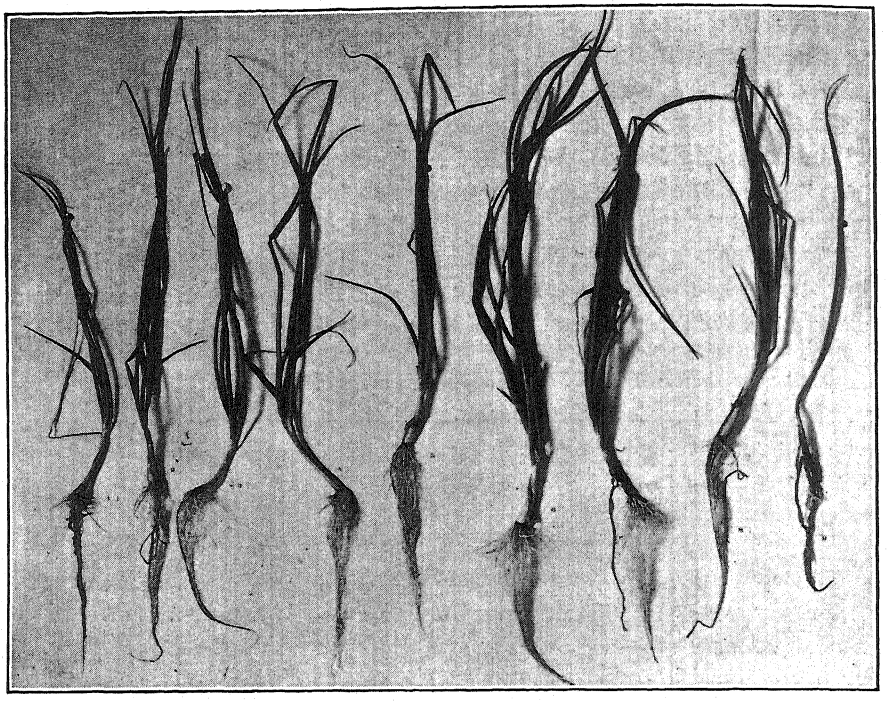


FIG. 4. Entire onion plants, one from each culture, representative of the average type. From left to right: pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0.

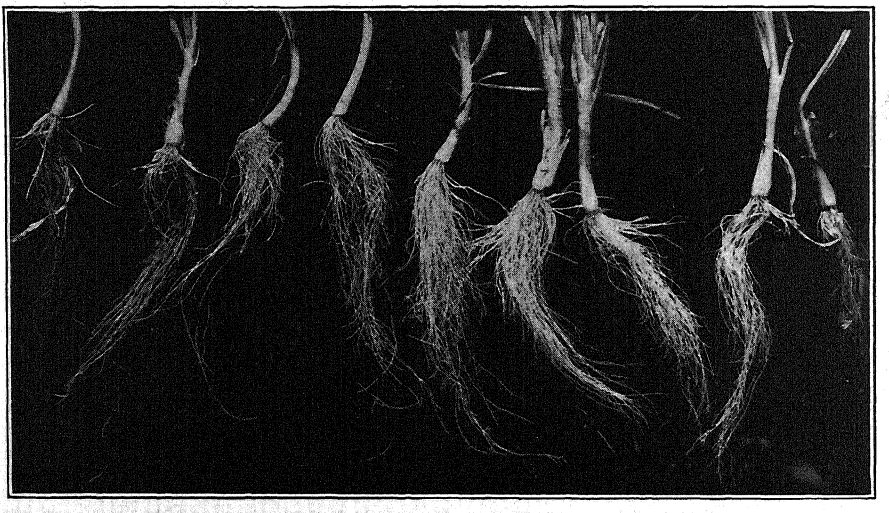


FIG. 5. The root system of onion plants, one from each culture, representative of the type. Cultures from left to right: pH 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 7.5, and 8.0.

The development of the root system and tops of the different plants seems to have been influenced considerably by the initial pH value of the culture solution (Figs. 4 and 5). This is shown in table 10 by the weights of the plants. These plants, 10 in each group, were dehydrated at 100° C. and their dry weight determined. The plants of series A were grown during the summer and those of series B during the winter months.

The different values obtained for the weight of the plants of the different cultures indicate that the H-ion concentration of the solution is responsible to a great extent for the differences in the growth of these plants. There is, however, a certain disagreement in the growth obtained in Series A and B at the same H-ion concentrations and the same culture solution. It seems that this difference is climatic, having to do more with sunlight and heat rather than with any other single factor, inasmuch as the plants were grown during the two different seasons. The results indicate that the optimum pH range for the growth of onions is between 5.5 and 6.5.

Growth of Onions in Soil Cultures of Different pH Values

The soil substratum was prepared, as mentioned before, with equal volumes of peat and Oakley sand and adjusted to different hydrogen-ion concentrations by means of 3 N H_2SO_4 or KOH. The H-ion concentration of the different cultures was determined on a Hildebrand electrode (12). The seedlings, germinated on sand, were as uniform as possible at the time of their transfer to the different cultures. The plants were allowed to grow from February 5 to May 25, 1922. The number of plants employed in each culture was 25, 5 plants to each 2-gallon stoneware jar. The results obtained are recorded in table 11.

TABLE 11.—*Weight, in grams, of green onion plants grown in soil cultures of different initial pH from February to May and at temperatures fluctuating between 50° and 110° F.*

Data taken	Series					
	1	2	3	4	5	6
Initial pH	3.6	5.0	6.0	6.8	7.6	8.0
Final pH	4.0	5.5	6.2	7.0	7.4	7.6
Weight in grams	500	1000	1300	1350	500	250
No. dead plants	10	0	0	0	15	20
No. etiolated plants	10	3	0	1	10	5
No. healthy plants	5	22	25	24	0	0

The behavior of onion plants in soil cultures is comparable to their behavior in culture solutions. The plants in both soil and solution cultures

either did not grow well or showed signs of distress at high acidity or alkalinity. It was particularly noticeable in soil cultures in which the titratable acidity or alkalinity was greater than that of the water cultures; in fact, some of the plants became etiolated and died. The optimum pH value for the growth of these plants was found to be between pH 5.0 and 7.0, this being almost the same as that reported for solution cultures. The final pH value of the different cultures varied somewhat from that of the original. There was an increase in pH value in those cultures that had an initial pH below 7.0, and a decrease in those whose initial pH was above 7.0.

Discussion of Results

The general belief, with respect to changes produced by plants in the reaction of their substratum, is that they tend to bring the final reaction nearer to the neutral point by the liberation of either acids or bases, as the case may be. Such conclusions have been based on observations made on culture solutions of more or less identical composition. With culture solutions of somewhat different composition it is possible that the same plant may behave differently.

The studies of Hoagland and Davis (14) on this subject are very enlightening. These investigators found that the H-ion concentration of plants transferred from a complete culture solution of pH 5.0 to a dilute solution of K_2SO_4, as well as to solutions of a number of other salts, increased from pH 5.0 to 3.2. They have suggested a hypothesis by which such phenomena may be intelligently explained. They state that the complexity of factors which influence the mechanism of absorption of the different ions from the solution is responsible for many of the changes of the initial H-ion concentration of the different cultures. They cite a number of examples which tend to emphasize the belief that under acid conditions anions are absorbed from the solution at a greater rate than the corresponding cations, providing that both are of equal valence. Under alkaline conditions the reverse reaction takes place. Bicarbonate ions (HCO_3) are considered to play the greatest rôle in the change of the initial H-ion concentration of the culture solution in either direction; *e.g.*, toward alkalinity or acidity. Under acidic conditions bicarbonate ions may replace the absorbed NO_3-ions, Cl-ions, etc., and form salts of a basic nature. Under alkaline conditions they may influence with their acidic properties the initial H-ion concentration of the culture solution. Theron's studies (38) on the influence of plant absorption on the initial pH reaction of the substratum bring out the same evidence as discussed above by Hoagland and Davis. Although Theron observed that wheat plants produced the best growth at pH 5.5, yet the culture which suffered least from changes in the reaction

had a pH value of 6.8. Pantanelli's (26) contention is that plants tend to maintain a favorable H-ion concentration by modifying objectionable reactions for best growth. Hoagland's (13) findings on barley strengthen the evidence, so often presented by many investigators, that different plants behave differently at different H-ion concentrations, barley thriving best at pH 7.0, whereas wheat thrives at 5.5. If Robbins' (28) interesting investigations on the isoelectric point of plant tissues establish definitely that plant tissues behave as ampholytes, then we may be able to understand better the type of reaction that takes place between the plant tissues, on the one hand, and the culture solution on the other.

There is no doubt that reactions of this type are responsible for the changes resulting in the initial H-ion concentration of the culture solution. The investigations of the writer (36) on the behavior of *Fusarium cromyophthoron* at different H-ion concentrations in culture media of carbohydrates, glucosides, and proteins, have shown that the changes that were produced in the pH value of the cultures above the isometabolic point of the particular substance were due to the liberation of organic acids. The changes, however, that had been produced in the reaction of the cultures of a lower pH value than the isometabolic point of the substance under consideration were due to the generation of HCO_3 with young cultures, and to NH_4OH with old cultures. Results obtained with fungi cannot be compared to those obtained with green plants owing to the great diversity in the nutritional requirements of the two groups of organisms.

The better growth produced by onion plants during the summer months in more acid cultures, as compared with that during the winter months, may be due to the greater photosynthetic activities of the plants, on the one hand, and to the availability of the different salts on the other. There is no doubt that plants are producing more organic matter during the summer than the winter months and that the availability of the majority of elements at more acid reactions is greater than at less acid or alkaline reactions, in spite of the fact that no precipitate of insoluble salts is apparent and the plants are grown in water cultures. High H-ion concentrations may, besides increasing the solubility of the various salts, hasten the escape of CO_2 accumulations in the culture solution, which, in turn, helps the diffusion of atmospheric oxygen and also prevents the precipitation of calcium. Higher acidities during the summer months—the busiest period for plants—may be more favorable for the development of plants. The question then arises: "Are we permitted to consider that there is only one pH value for the best development of plants during both winter and summer months?" The answer to this is no!

The changes in the initial H-ion concentration of the different soil cultures were possibly not produced by the plants grown therein, but by the various acidic and basic substances of the soil. The results indicate that

those cultures with an initial pH value lower than 7.0 had a higher final value, and those with a higher initial value had a lower final pH. Gimingham (6) found that similar changes were produced in soils left in contact with water and then exposed to air for some time without green plants. He did not attempt to exclude microorganisms.

THE INTERRELATIONS OF FUSARIUM CROMYOPHTHORON AND ALLIUM CEPA IN THE DEVELOPMENT OF THE PINK-ROOT DISEASE

Symptoms of the Disease

Roots are the plant organs that are more frequently invaded and killed by the causal organism (Fig. 6). A pink pigment is formed within the invaded tissues; hence the name pink root. The invaded roots die in the majority of cases. The cortical tissue undergoes decomposition leaving behind the exodermis and stele of the root. The pink pigment may be detected on the dried roots of onions sold in the market as well as on those of onions in the field. The pigment in dried roots is not of an intense color, but it can be brought out merely by wetting the tissues.

Nature of the Pink Pigment

As the development of pigment in diseased roots was not well understood, certain experiments were carried out to furnish the necessary information. Onion scales, some of which were taken from the center and others from the periphery of the bulb, and roots were subjected to various treatments of acids and bases with the object of forcing out the manifestation of their pigments. The reagents were heated in every case to 80° C., and the tissues then immersed in the solution for two minutes. The results obtained from these treatments are recorded in table 12.

TABLE 12.—*Influence of acids and bases on the development of pigment in onion scales and roots, when exposed to indirect sunlight before treatment and when treated immediately after detachment from the bulb*

Reagent and concentration	Hours of exposure to light	Presence of pigment on	
		Scales	Roots
0.1 N HCL	0	–	–
do do	24	+	–
10% Oxalic acid	0	–	–
do do	24	+	–
do Malic acid	0	–	–
do do	24	+	–
do Tartaric acid	0	–	–
do do	24	+	–
do Citric acid	0	–	–
do do	24	+	–
1.0 N NH_4OH	0	–	–
do do	24	–	–

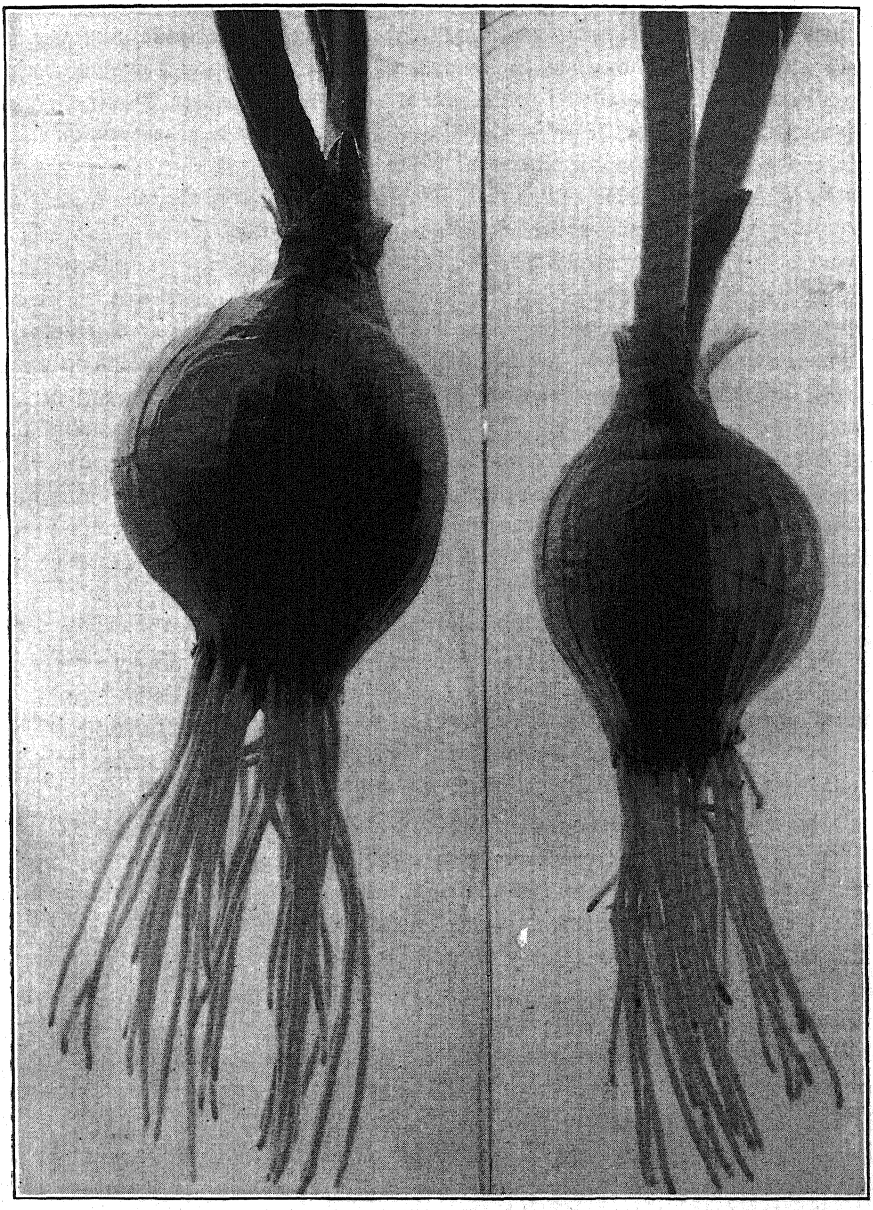


FIG. 6. Left: Plant grown in sterile water and inoculated with *F. cromyophthoron*, showing the initial development of pink root symptoms in the root tips. Right: Plant growing in sterile water and inoculated with *F. cromyophthoron* showing advanced development of pink-root symptoms on root tips.

The tabulated results indicate that acids brought out the pink pigment of onion scales previously wounded and exposed to indirect sunlight for 24 hours, but failed to do so in the case of roots that had received identical treatment. The peripheral scales of the onion yield quite readily a pink pigment if treated with acids alone, and without any exposure to sunlight after wounding. The formation of pigment in lily bulbs has been investigated by Mirande (22), who says that oxidation and acidification take place before pigmentation. He has demonstrated a strict localization of an oxidase in those cells which produce anthocyan pigment. In scales of lily bulbs, which were detached and exposed to the light, there was a general acidification due to wounding and a special acidification correlative with pigmentation. The negative results obtained by the action of acids on onion roots indicate that these organs lack either the oxidase enzyme or the anthocyan pigment or both. The development of the pink pigment of diseased roots may be attributed to the parasitic organism. Fusarium species produce various pigments on artificial as well as on natural media, the hues of which may vary with changes in the pH value of the surrounding solution (33).

Inoculation Studies in Water Cultures

The onion seedlings used for inoculation purposes were germinated in sterile sand. The same method was used for the germination of bulblets. Both seeds and bulblets were disinfected previous to germination with an alcoholic solution of mercuric chloride. Certain lots were disinfected with formaldehyde.

The seedlings were transferred either to water cultures or to soil cultures. The water cultures were composed of the salts mentioned in the first part of this paper and soil of equal volumes of peat and Oakley sand. The soil cultures were sterilized with formaldehyde.

The seedlings that developed from bulblets were, in certain cases, freed from the scales of the bulblet before planting and, in other cases, left undisturbed.

Pure cultures of *Fusarium cromyophthoron* were placed in contact with the root system of the seedlings at the time of their transference from the germinating bed to the water and soil cultures.

The plants in the water cultures were examined at rather frequent intervals and the developments recorded. The plants in the soil cultures were examined only at the termination of the experiment. The results that were obtained with onion seedlings in both water and soil cultures and under different treatments are recorded in tables 13–17. The pink root symptoms are recorded in percentages. These numbers refer to roots in each group of

10 plants. To illustrate: If in a culture of 10 plants, there are 100 roots regardless of the number per plant and if only 3 out of the total of 100 show symptoms of the disease, the results would be recorded as 3 per cent.

TABLE 13.—*Percentage of roots with symptoms of pink-root disease produced on onion plants freed from the bulblet scales at the time of planting and grown in water cultures of different initial H-ion concentrations*

Initial pH of cultures	Appearance of pink root symptoms during growth				Average wt. of roots grams per plant
	Planted Jan. 20	Inoculated Feb. 8	Mar. 8	Mar. 20	
4.0	None	None	15% pink	20% pink	1.0
4.5	do	do	5 do	5 do	1.5
5.0	do	do	2 do	3 do	2.5
5.5	do	do	1 do	1 do	3.0
6.0	do	do	0 do	0 do	3.2
6.5	do	do	0 do	0 do	3.2
7.0	do	do	0 do	0 do	3.0
7.5	do	do	10% yellow	15% yellow	1.5
8.0	do	5% yellow	15 do	20 do	0.75

TABLE 14.—*Percentage of roots with symptoms of pink-root disease produced on onion plants planted with the bulblet scales attached and grown in water cultures of different initial H-ion concentrations*

Initial pH of cultures	Appearance of pink root symptoms during growth				Average wt. of roots grams per plant
	Planted Jan. 20	Inoculated Feb. 8	Mar. 8	Mar. 20	
4.0	None	None	20% pink	30% pink	0.8
4.5	do	do	15 do	25 do	1.2
5.0	do	do	5 do	10 do	2.0
5.5	do	do	3 do	5 do	2.6
6.0	do	do	1 do	1 do	3.0
6.5	do	do	0 do	1 do	2.8
7.0	do	do	0 do	0 do	2.4
7.5	do	5% yellow	15% yellow	25% yellow	1.0
8.0	do	do	25 do	40 do	0.3

The results obtained in the different experiments indicate that at high H-ion concentrations the development of pink root symptoms is more pronounced than at low H-ion concentrations. This does not mean that plants grown at higher pH values than 7.0 made a wonderful growth; on the contrary, they were stunted more by the effect of OH-ion concentrations than

TABLE 15.—*Percentage of roots with initial and final symptoms of pink-root on onions grown in water cultures of different H-ion concentration from May 1 to June 15, 1923, in the greenhouse. Seedlings inoculated in seedbed, or otherwise, as stated*

Culture	Symptoms	Percentage of infected roots in water cultures of different initial H-ion concentration						Inoculation
		pH 4.0	pH 5.0	pH 6.0	pH 6.5	pH 7.0	pH 7.5	
1	Initial	25	25	25	25	25	25	Seed-bed
	Final	75	75	50	50	40	35	
2	Initial	50	50	50	50	50	50	do
	Final	100	75	100	75	70	70	
3	Initial	75	75	75	75	75	75	do
	Final	100	100	100	100	90	90	
4	Initial	0	0	0	0	0	0	Trans-
	Final	15	5	1	0	0	0	planting
Control	Initial	0	0	0	0	0	0	
	Final	0	0	0	0	0	0	

TABLE 16.—*Percentage of roots with symptoms of pink-root disease produced on onion seedlings, after inoculations with pure culture of Fusarium cromyophthoron, grown in water cultures of different initial pH, at temperature 20° to 34° C. Inoculated May 10, 1923*

Culture	Symptoms	Percentage of infected roots in water cultures of different initial H-ion concentration					
		pH 4.0	pH 5.0	pH 6.0	pH 6.5	pH 7.0	pH 7.5
1	May 25	5	1	0	0	0	0
	June 17	15	5	1	0	0	0
2	May 25	7	0	1	0	0	0
	June 17	20	15	3	1	0	0
3	May 25	2	0	2	0	0	0
	June 17	25	25	5	2	0	0

the plants grown at a lower pH. The pink pigment did not develop on roots grown in cultures of high pH value, but a yellow pigment developed instead.

A comparative analysis of the results obtained with different planting material and recorded in tables 13 and 14 indicates that the plants that had been freed from some of their scales developed less pink root than those that

TABLE 17.—*Condition of onion seedlings, grown in water cultures of different initial pH, and inoculated with Fusarium cromyophthoron, at temperatures from 20° to 34° C.*

Condition of plants	Percentage of normal, stunted, chlorotic, and dead onion plants grown in cultures of different initial H-ion concentration					
	pH 4.0	pH 5.0	pH 6.0	pH 6.5	pH 7.0	pH 7.5
Normal	15	95	100	85	30	10
Stunted	80	3	0	10	30	10
Chlorotic ...	0	0	0	1	25	50
Dead	5	2	0	4	15	30

were planted with the superficial bulblet scales on. The scales apparently furnish an excellent medium for the saprophytic development of the fungus, thus increasing the inoculum and chances of root infection to a considerable degree.

Table 15 contains the results obtained on onion seedlings inoculated in the seedbed long before transferring to cultures of different hydrogen-ion concentration and also those obtained on plants that were inoculated after transplanting. These results indicate that various concentrations of H-ions had no beneficial effect whatever on seedlings inoculated in the seedbed although they had some influence on the development of symptoms on plants inoculated after transplanting. It may be stated, therefore, that plants already infected with the pink-root disease and submitted to treatments of different H-ion concentrations do not seem to show a reduction of pink-root symptoms, unless new roots are produced that may evade the parasite under very favorable conditions. The color of the pigment on the roots is somewhat different in acid and alkaline solutions, being rose pink in the former and purplish pink in the latter. The slight increase of pink-root symptoms on plants in cultures of pH 7.0 and 7.5 (Table 15) presents a condition that has never been observed before by the writer and is rather difficult to explain. The opinion of the writer, however, is that these tissues that showed an increased development of pink-root symptoms may have been diseased at the time the plants were being transplanted, but had not yet, for reasons not well understood, produced the pink coloration.

Tables 16 and 17 give an account of the percentages of roots with symptoms of pink root produced at different H-ion concentrations and the condition of the plants at the same time. The death rate is higher under high pH values, namely, 7.0 and 7.5; whereas, the development of pink-root symptoms is nil. The death of plants under such conditions may be at-

tributed to the toxic effect of high concentrations of OH-ions. The chlorotic condition developed at high pH values may be attributed to the precipitation of ferric-ions. The stunting of plants grown in cultures at pH 4.0, 7.0, and 7.5 may be due, in the first case, to high concentrations of H-ions and, in the last case, to OH-ions, or lack of certain ions. The development of pink symptoms does not necessarily presuppose the death of the infected plant. Pink-root symptoms associated with certain roots may signify the death of those individual roots, but not of the entire plant. The entire plant may die under certain conditions, but the death rate hardly ever exceeds 10–15 per cent of the infected plants. Stunting more commonly results in plants infected with pink-root disease.

Inoculations in Soil Cultures

The soil was prepared of equal volumes of Oakley sand and peat and adjusted to different pH reactions by additions of different amounts of H_2SO_4 and KOH. It was sterilized with an application of about 2 per cent formaldehyde. Although the soil was not completely sterile, it was free from many objectionable organisms. No attempt was made to keep it free from other organisms, such as those contained in the air or the water used for irrigation after planting. The inoculum, *F. cromyophthoron,* was introduced in the soil in the same hole in which the young plant was placed at the time of planting. The growth of these plants extended from February to July, with temperature fluctuations varying between 14° and 34° C.

The plants grew fairly well in all the different soil cultures, except for the slight stunting that developed in some of them. Some deaths were noted in a few cases. At the termination of the growth period the different plants were removed as carefully as possible from the containers to avoid breaking or loss of any of the roots. The roots were segregated in two distinct groups, the primary and secondary (34), and were studied carefully as to the amount of pink-root symptoms, etc. The findings are recorded in tables 18 and 19.

The evidence presented in these tables indicates that the development of pink-root symptoms may or may not be correlated with parasitism. The fact that pink-root symptoms appear, for the most part, on primary roots after the bulbing period of the plant indicates that relationship of the fungus to the root tissues is of a saprophytic nature, the tissues having previously died from natural causes. The association of the fungus with primary roots may then be considered saprophytic in about 75 per cent of the cases. Parasitism should be restricted to cases where the fungus is found in association with apparently healthy secondary roots showing pink symptoms.

TABLE 18.—*Percentages of primary and secondary roots with symptoms of pink root disease on onion seedlings grown in soil cultures of different initial pH, condition of the roots and readings of the initial and final pH of the soil solution*

pH readings of soil cultures		Percentage of infected roots		Condition of roots (percentage dead)	
Initial	Final	Primary	Secondary	Primary	Secondary
3.5	3.8	10	5	100	25
5.0	5.5	100	1	100	2
6.0	5.9	100	0	100	0
7.0	6.8	50	0	100	0
7.5	7.3	3	0	90	10
8.0	7.6	0	0	100	80

TABLE 19.—*Condition of onion seedlings grown in soil cultures of different initial pH and inoculated with a pure culture of Fusarium cromyophthoron, at temperatures 20° to 34° C.*

Condition of plants	Percentage of normal, stunted, chlorotic, and dead onion plants grown in cultures of different H-ion concentration					
Initial pH Final pH	3.5 3.8	5.0 5.5	6.0 5.9	7.0 6.8	7.5 7.3	8.0 7.6
Normal	10	90	100	85	20	0
Stunted	85	8	0	15	60	40
Chlorotic ...	0	0	0	0	30	20
Dead	5	2	0	0	20	40

The development of two sets of roots by onion plants (34) complicates considerably the diagnosis of purely parasitic from saprophytic cases. The death of the primary roots is believed to result from certain histological complications. The association of these roots with pink-root symptoms does not represent parasitism in the true sense of the word.

It has been found, as far as the influence of different concentrations of H-ions on plant growth is concerned, that the influence is the same whether soil or water cultures are involved. The influence of the H-ion concentration on the development of pink-root symptoms is likewise the same whether it applies to water or soil cultures.

The few cases of pink root that have been observed in somewhat alkaline soils may be due possibly to the low moisture content of such soils. The fungus is capable, without any doubt, of producing local changes in tissues not bathed with great quantities, or a continuous film of water, which helps

the free movement of the hydrogen of hydroxyl ions of the soil solution to or from an area. Such reactions may be produced by the by-products of metabolism of the fungus which may be acidic or basic in reaction. In alkaline soil cultures of relatively high moisture content the development of pink root has never been observed by the writer.

The Disease in the Seedbed

Soil of the same type as mentioned before was used for the germination of onion seeds. The pH of this soil was 5.2. Two agate pans $25 \times 35 \times 5$ cm. were used for the growth of the plants, one of which was inoculated with *F. cromyophthoron* at the time the seeds were sown and the other left for control.

The plants in both cultures started to germinate on the tenth day. Symptoms of the disease appeared on the roots after the twenty-fifth day in the inoculated culture; many of the plants also wilted and died.

It was noticed that the severity of the disease, as far as the death of individuals was concerned, could be minimized by a lowering of the moisture content of the seedbed. Nearly 100 per cent of the surviving plants showed symptoms of pink root at the end of 70 days. About 80 per cent of the total number of plants died by the end of this period. The results illustrated in figure 7 show the inoculated pan with the smaller number of plants compared with those in the noninfected pans. The indications are that onion

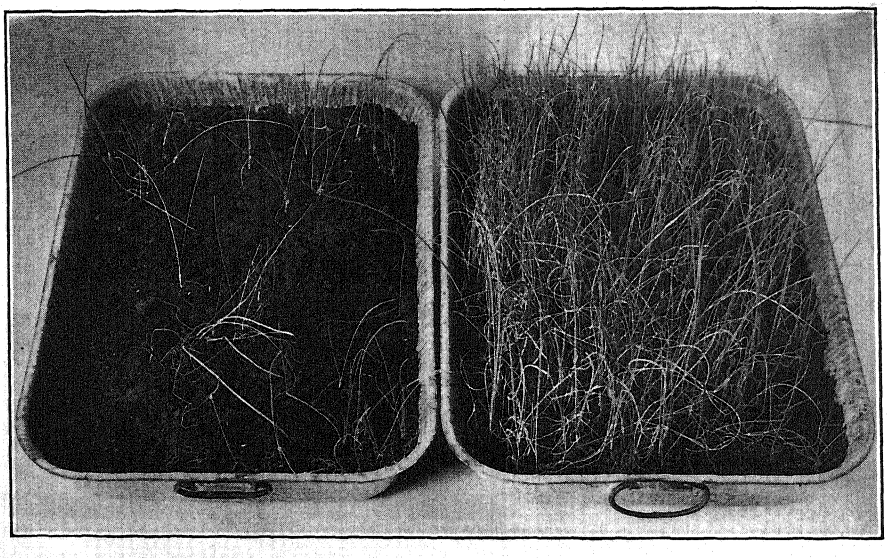


FIG. 7. Left: Onion seedlings inoculated with *F. cromyophthoron* at time of sowing. Right: Onion seedlings not inoculated, but kept for control.

seedlings are very susceptible to pink root, their resistance increasing with age. It is possible to produce almost 100 per cent infection but not death, providing temperature, moisture, and soil acidity are favorable.

DISCUSSION OF RESULTS

Observations made on the development of pink-root symptoms in a number of inoculated onion plants have indicated that the tissue adjacent to the tip of the root is the first to develop pink-root symptoms. Lateral roots may also develop symptoms on the same root area. The pigment, a characteristic symptom of this disease, is the result of the metabolic processes of the fungus on the root tissues. The fungus enters the root of the host plant through the tissues which are least resistant. Such tissues occur near or at the tip, being of embryonic nature and not having undergone differentiation or high specialization. These tissues are neither shielded by the rootcap proper nor protected by such resistant substances as lignin, or suberin. The cementing substance of the cells of the tissues under discussion, being of pectin, is highly susceptible to the hydrolyzing effect of pectinase. Whether the fungus makes its initial invasion into the root by mechanical pressure (3), by means of hydrolytic enzymes, or some other less understood means, it is difficult to say. After the initial invasion the various steps of fungal invasion into the tissues of the host may be easily followed. It usually disintegrates the cortical tissue and all embryonic tissues, leaving intact those tissues that have undergone considerable differentiation. The tissues that are usually left undecomposed on an onion root which shows symptoms of pink root are the exodermis and stele. The pigment is deposited more often on the stele than on the exodermis.

The fungus may invade the tracheids of the root, causing wilt and occasionally death of the young plants. The fungus is not limited in its invasion to the roots alone. It may enter the stem or bulb through the roots, and produce, under proper conditions, disintegration and death of the bulb.

The influence of the H-ion concentration of the solution on the development of pink root deserves consideration in more than one way. The H-ion concentration of the culture solution may influence favorably or unfavorably the development of onion plants as follows: (*1*) H- or OH-ions may either increase or decrease the availability of other ions, and (*2*) high concentrations of either H- or OH-ions may cause injury through their toxic properties. Repeated observations by the writer of the root tips of a number of plants subjected to high concentrations of either H- or OH-ions have shown that such parts of the plant were killed outright. Addoms (1) has also observed that in the roots of wheat plants subjected to H-ion concentrations ranging in acidity between pH 3.47 and 3.94 the protoplasmic contents of the cells were coagulated and flocculated.

In regard to the influence of H-ion concentrations on the growth and parasitic behavior of the fungus, the evidence presented so far, indicates that the fungus is capable of resisting H-ion concentrations as high as pH 3.0 and as low as pH 10.0. Both limits exceed those that onion plants can tolerate. H-ion concentrations ranging between pH 3.5 and 7.5 have no influence whatever on the release of pectinase by the fungus. The writer (35) has found that pectinase may be released by very old and possibly dead hyphae but not by young and healthy hyphae. The fungus therefore may make use of mechanical pressure rather than of pectolysis as an initial step for the invasion of the onion root tissues. Other means, such as the killing of the tissue by toxins, accidental wounds, etc., may open a path to fungal invasion.

Considering temperature as a possible factor for increasing or decreasing pink-root infections, the findings indicate that many more cases of pink-root occur during the warm than in the cool months of the year. Jones and Tisdale (18) have found that higher temperatures increased the rate of infection in flax wilt, and Clayton (4) has reported similar behavior for tomato wilt. The influence of temperature on the development of diseases caused by Fusaria might be of double nature. First, it may stimulate growth in the fungus, which in turn may increase its numbers and also the chances of infection. Second, it may inhibit the normal development of the host, which may result in loss of vitality and susceptibility to infection.

In regard to the occurrence of the pink-root disease in the seedbed, the results are in accordance with those of other investigators working with different species of *Fusarium*. Rose (30) observed, in germination tests of 18 varieties of wheat, that a mortality of from 3 to 98 per cent could be produced by *F. culmorum* var. *leteius* Sher. Hartley (11) found, likewise, that *F. moniliforme* and *F. ventricosum* Ap. et Wr. were pathogenes of the seedbed.

In conclusion, it is the writer's opinion that the presence of pink root symptoms alone on dead roots of the host and the isolation of *F. cromyophthoron* or any other species of *Fusarium* are not sufficient evidence for reliable diagnosis of pathogenicity. The occurrence of pink-root symptoms on apparently healthy or diseased young primary or secondary roots and the presence of the fungus inside the host tissues should be the only reliable criteria for determining pathogenicity.

SUMMARY

The physiological behavior of *Fusarium cromyophthoron* Sid. in cultures of different H-ion concentrations may be summarized as follows:

The growth of this organism is influenced appreciably by the reaction of the culture medium, the total sugar content, and the age of the culture. Best growth was produced at about pH 6.0, although pH values as high as 7.5 and as low as 3.0 did not stop the growth of the fungus.

The initial pH value of the culture solution was changed in the majority of cultures by the metabolic by-products of the organisms, these being acidic and basic in nature. The cultures that suffered the least in their initial pH were those that had a reaction as near as possible to the isometabolic point of the principal nutrient substance of the culture.

The consumption of dextrose was considerably less at pH values lower than 4.0. This is considered to be the isometabolic point for dextrose. Above this pH value the consumption of sugar reached its maximum. Whether the high H-ion concentrations had anything to do with the electric charge of the sugar molecule, or whether a reversal of the electric charge of the proteins of the fungus mycelium had taken place, inhibiting the absorption of sugar molecules at low pH values, the writer is not in a position to state definitely.

After the fungus has absorbed all of the available sugar of the culture medium, its mycelium undergoes autolysis. During this stage the reaction becomes decidedly alkaline owing to ammonia released from the hydrolyzed protoplasmic proteins of the fungus. Autolysis under relatively acid conditions, that is, below pH 4.0, is negligible, or may develop very slowly because of the slow consumption of the sugars of the culture.

Temperature influences vary appreciably the growth of the fungus. A temperature of 28° C. was found more favorable for the growth of this organism than one of 20° C.

The effect of H-ion concentration on the growth of onion plants was found to be as follows:

Onion plants grow best in both water and soil cultures at pH values between 5.5 and 6.5. Higher or lower pH inhibited their growth somewhat.

Onion plants change the initial reaction of their culture solution. The least changed culture was that with an initial pH of 6.5. It is believed that such changes were brought about by the uneven absorption of equivalent ions in the cultures having a reaction above or below pH 6.5. High concentrations of H- or OH-ions were found toxic, the latter being more toxic than the former.

Differences of temperature and sunlight may shift upward or downward the pH scale for the optimum growth of plants. It was found that during the summer months a slightly more acid reaction is more favorable for the growth of onions than in the winter months.

The pathogenicity of *F. cromyophthoron,* measured by the death rate it causes, is higher at pH values above 6.5; whereas, the development of pink-root symptoms is inhibited. The fungus at pH values lower than 6.5 may invade naturally dying roots or may even kill some and produce the typical symptoms of the malady, but it is not capable of wholesale invasion of the roots and death of the plant. At pH values above 6.5, however, the death of roots and plants is more common, although the production of pink-root symptoms is nil.

The pigment characteristic of the pink-root disease is only produced by the fungus at low pH values, namely, between pH 3.5 and 6.0. The invasion of roots is more extensive at a higher pH. The greater death rate of roots grown in cultures between pH 6.5 and 8.0 is believed to be due to the toxicity of high concentrations of OH-ions to the tissues of the host; this condition decreases their resistance considerably to invasion by the fungus.

It is unsafe to draw conclusions on the pathogenicity of *F. cromyophthoron* when associated with the dead tissues of the host. As onion plants produce more than one set of roots, it is possible that the old ones may die of senility, or lose their resistance on account of senility, and become invaded by *F. cromyophthoron* or other facultative parasites. The death of roots under such conditions is not due to the aggressive parasitism of the fungus, but to more than one factor. If, on the other hand, the fungus is found associated with a recently invaded healthy root, then one is justified in associating the death of such a root or roots with *F. cromyophthoron.*

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SOME OBSERVATIONS UPON THE WHITE PINE BLISTER RUST IN NEW YORK

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In 1923 the writer began some studies of the white pine blister rust in New York for the purpose of obtaining information regarding damage and kindred subjects, for the use of the New York Conservation Department in its campaign of blister rust control. The work was done under the direction of Dr. H. H. York, Forest Pathologist of that Department. Some preliminary data obtained upon the damage caused by blister rust in New York appeared recently (13). The present paper gives the results of certain observations incident to the damage studies.

FUNCTIONS OF THE DIFFERENT SPORE STAGES IN THE LIFE CYCLE OF CRONARTIUM RIBICOLA

When investigation of the blister rust was begun in earnest in 1916, the prevailing idea of the respective parts played by the different spore forms of the fungus was approximately as follows: The aeciospores do little more than initiate the rust by infecting *Ribes* in the spring; the urediniospores formed on *Ribes* are blown a considerable distance and cause new infections, which develop another generation of urediniospores likewise to be blown far and wide, and so on for several generations; and the sporidia perhaps also are able to disseminate the fungus in somewhat extended flights to the pine. The idea was that all these spores disseminated the fungus somewhat widely, with the greater part of the spread accomplished by the uredinial stage because of its repeating character. Indeed, the observations made in scouting for the rust in the southeastern part of the New England States in 1916 left the impression that the disease was spreading very rapidly by means of the long-distance carriage of urediniospores (14, p. 56).

Studies made in the next two years, however, cast doubt upon this idea of the rôles played by the different spore stages, and, although blister-rust workers in general have for some time had a correct idea of the situation, it apparently has not been presented to pathologists in general.

The writer's first intimation of a different conception of the functions of the various spore stages came during the course of some work which he carried out in 1918 near Lewis, Essex County, New York, for the Office of Forest Pathology, United States Department of Agriculture. It was found, for example, that the supposed disseminating stage, the urediniospores, did not disseminate the rust at all, but merely intensified the infection locally within very narrow limits (12, 14). A study of the different spores gave

the clue, and other studies by the writer led to the formulation of a different scheme, presented in manuscript form to the Office of Forest Pathology in 1918. Further work by the writer and others in that Office, and also by the writer for the New York Conservation Department, recently have substantiated and elaborated the following general scheme.

The aeciospores of *Cronartium ribicola* are dry, dusty, powdery, and not easily wet. The spores are produced in quantities like windblown pollen, with a moderate ability to hover in the air (5), and capable of long-distance dissemination by the wind. There seemed to be no reason why these spores could not be carried miles as easily as hundreds of feet. This long-distance dissemination was indicated by some evidence produced by McCubbin (6), Posey and York, Overholts, and the writer (12, 14), and was satisfactorily proved by Pennington (7, 9).

The urediniospores of this fungus, on the other hand, are moist, sticky, easily wettable, usually found in clumps when collected by aspirators, megaphone traps, glass slides, etc. They are not produced in quantity, are never seen in clouds visible in ordinary daylight, and are seen only as sparse points of light by the beam-of-light method. They do not hover long, but appear to sink relatively rapidly (5, 14). They are easily separated from each other, spattered about by rain, and disseminated by insects (11, 12, 14). They have the general characteristics, including the spiny coat, of insect-carried pollen. Studies made during the past few summers for the Conservation Department in New York have substantiated the observations of 1918, that urediniospores are disseminated for only relatively short distances under ordinary conditions. In fact, the writer in New York recently has been unable to find good evidence of distance dissemination of these spores, although in very moist seasons urediniospores from *Ribes nigrum* may be blown 2,000–3,000 feet. The urediniospores are often disseminated no farther than within the original bush or to neighboring bushes. Under normal weather conditions, in any infected *Ribes* bush, there can usually be found distinct generations of uredinia, including what is apparently the first generation resulting from the aeciospore infection (12).

The only change in the original conception of the function of the sporidia of *Cronartium ribicola* has been a tendency to shorten the distance they may be blown. They apparently can disseminate the rust for distances up to one and one-half miles, perhaps farther in extreme cases, but ordinarily not more than half a mile, and can cause damage of considerable amount only at little more than 500 ft. in most cases. The main factor determining the distance these sporidia are disseminated seems to be that of number alone, following the mathematical laws of chance. The more sporidia produced, the greater will be the chance that some will be carried to considerable distances. The danger of *Ribes nigrum* to nearby pine

stands lies in the abundance of sporulation, the myriads of sporidia produced by the fungus, on this host.

These studies have resulted in the following conception of the functions of the spore stages of *Cronartium ribicola*:

(1) The aecial stage not only initiates the cycle but, more important, is primarily the long-distance-dissemination stage.

(2) The uredinial stage is a local-intensification stage, a repeating stage, in which spores are formed that are incapable of long-distance dissemination but which produce an abundance of lesions locally, for the purpose of increasing the volume of telial material as a basis for the production of sporidia for infection of the pine.

(3) The telial stage is a combination of an emergency stage, which insures the continuance of the rust cycle by developing just prior to the death of the host tissue, and a multiplication stage, whereby the fungus apparently puts forth its entire available energy in producing a multi-seriate column of teliospores, each of which multiplies itself again in the formation of four sporidia.

(4) The sporidia are the agents of local, limited dissemination, but their chief function is the reinfection of pine to complete the cycle.

BLISTER-RUST YEARS IN THE ADIRONDACKS

It has been shown that most of the infections in the Adirondacks took place in 1911, 1913, 1916, and 1919 (13). Nearly half of the cankers were found to be upon 1919 wood; and in view of the fact that there were more cankers on 1918 and 1917 wood than wood of other years before or since 1919, it appeared that in all probability most of these cankers resulted from infections in 1919 in two- and three-year-old needles, even though artificial inoculations of three-year-old needles had proved unsuccessful (16). If such were the case, as seems likely, between 60 and 70 per cent of all cankers in the Adirondacks resulted from infections in 1919, a year known to have been unusually favorable for development of the blister-rust fungus. A study of the distribution of cankers by years shows the progress of the fungus as it becomes established, is disseminated, and increases in amount slowly and gradually, gathering momentum in the relatively unfavorable years, and literally showering the pines with an immense volume of sporidia in the favorable years.

The first really big blister-rust blast occurred in 1919. In previous years there had been nothing approaching it in severity, but it is beginning to appear now that the epidemic in some of the recent years may approach or even exceed it in places. This of course was to be expected.

If relative humidity is a factor in producing a severe blister-rust attack (15), it was expected that 1924 might be added to the list of years of heavy

blister-rust infections. There were, however, certain factors during 1924 operating against abundant infection of pine. The principal one was cold weather in the spring and early summer, which delayed the general infection of *Ribes* until late in July and the production of the telia in any considerable quantity until the last of August. Under ordinary conditions, in a moist season in the Adirondacks, the *Ribes* would be generally infected by the first of July; during July and August there would be a great increase in the uredinial stage of the rust, and an abundance of telia and sporidia after the first of Auguust. In 1924, however, the production of telia was decidedly limited. Observations in 1927 indicated that little infection took place in 1924.

It was predicted that 1925 would be another epidemic year. There was an unusually copious production of aeciospores in the spring, and the summer was rather moist and reasonably cool, especially during the period of telial production. During the latter part of the summer, teliospores were produced in abundance, and the stage appeared to be set for an epidemic.

Observations made in the early part of 1925 confirmed the general predictions. There are parts of the Adirondacks (especially the northern portion) where there was only a moderate amount of pine infection in 1925, but in other sections it appears that ultimately it may be proved that the epidemic of 1925 was more severe than that of 1919.

The summer of 1926 was rather dry and few infections of this year have been found. On the other hand, in 1927 there was an unnusual amount of precipitation in the Adirondacks, which provided several prolonged periods of high humidity favorable for infection of the pine (See 15). It is therefore expected that 1927 may also be looked back upon within the next few years as another one of the years of unusually heavy pine infection.

HISTORY OF BLISTER-RUST INFESTATIONS AS APPLIED TO CONDITIONS IN THE ADIRONDACKS

Inasmuch as 1919 was the year of greatest infection in the Adirondacks —at least as far as known definitely up to the present—there have been suggestions of the possibility that blister rust has reached the peak of its damage in that area. It may be possible that 1919 will prove to be the year of greatest pine infection in blister-rust history, although there is now room for considerable doubt as to this. But even so, the peak of the damage is quite another thing. It is difficult to conceive of such a thing so long as aeciospores are formed each spring, while *Ribes* exist near pine, and while seasons favorable for infection of pine occur periodically.

An attempt has been made to outline the history of blister rust in any given locality (13). The introduction of the disease by one or more wind-

blown aeciospores is called the first critical point. Following the introduction of the disease comes the intensification of the original aeciosporic infection by the uredinial stage (the local intensification stage) and, sooner or later, of course, infection of the local pines. Following local establishment on pine, a gradual increase in the amount of rust from year to year is inevitable until there is enough rust on pine to cause a general and heavy initial infection of *Ribes*, if not each year, at least in some very favorable year. This point is called the second critical point in the blister-rust history of a locality, and is attained as a result of dissemination and multiplication by the aecial stage and to a large extent by the intensive multiplication by the uredinial stage.

The third critical point is the initiation of the waves of infection which follow the general infection of the pine as above mentioned, when only favorable conditions for infection are required for great damage to pine. Each of these waves of infection does great damage to the pine; the younger the trees the more damage. The trees infected by the first great wave may or may not be damaged by subsequent waves, but infection at the earlier ages is always more likely to be fatal. Even though subsequent waves do no more damage to the trees as they become older, the young seedling reproduction continues to suffer with each succeeding wave. A peak in the damage can not be said to have been reached under these conditions.

The fourth critical point is the stage in which maturing trees begin to die from blister-rust attack.

The subsequent history of blister rust is a continuation of the destruction of trees of all sizes, even the oldest and over-mature, unless the matured trees are lumbered. As far as known, the momentum of this destruction continues as long as there are white pines to be killed, *Ribes* to carry the infective material, and conditions favorable for infection of the pine.

There is no evidence of acquired resistance or immunity of any sort in the pine or in any other gymnosperm. It is expected, as well as hoped, that some of the angiospermous trees such as the chestnut will develop a resistance to their diseases, but whether the more primitive gymnospermous organization could respond in such a way is more questionable.

One factor in the blister-rust complex, however, that may have considerable bearing upon the checking of the progress elaborated above is biological control. The rust fungus in the cankers on pine dies when the tree dies or the branch is shaded out. It may also die when it kills what appears to be hypersusceptible host tissue. The cankers on pine are sometimes destroyed in considerable numbers by rodents and other animals (14), and possibly by secondary fungi. The volume of aeciospores is certainly considerably reduced in certain seasons by these means, although it is probable that even

a severe reduction in the aecial stage would not affect the ultimate volume of sporidial production to an appreciable extent. The general functions of the uredinial and telial stages in the life cycle, as outlined above, seem adequate to provide for such an emergency. A few aeciospores, relatively speaking, can initiate a tremendous amount of blister rust in a season.

A more important phase of biological control concerns the *Ribes*. Inasmuch as *Ribes* are necessary for the spread of blister rust, anything which affects them must necessarily affect the amount of the disease on pine. The total potentially infectable leaf surface of *Ribes* may be seriously reduced by defoliation by the blister-rust fungus or by insects. Defoliation may be partial or complete, may be repeated once or twice a season, and may be repeated in two or three successive seasons. Successive defoliations during two or three seasons, or perhaps in one season, usually result in the death of the bushes (8). There are many stands of seriously-infected pine near which there are no *Ribes* because they undoubtedly had been killed in one way or another after the pines were infected. In the Kelm Mountain plot in 1924, it was found that 50 per cent of the existing footage of stem was devoid of leaves, and half of this, or 25 per cent of the total footage, was dead. In 1927, between 50 per cent and 75 per cent of the leaf-bearing stem of the *Ribes* was dead. At the Dannemora plantation in 1927, the cankers were for the most part from 1919 and 1921 infections with very few on 1925 wood. It was found that the bushes of *Ribes triste,* from which the infection came, bore only 6–15 leaves each on young and very short stems, and yet had very extensive and well established root systems. The original bushes had apparently been seriously damaged by blister rust, insects, or by other means.

Therefore it may be possible that the momentum of the white pine blister-rust depredations may be checked considerably by destruction of the Ribes; at least their elimination may mean periodic variations in severity of the rust and perhaps a permanent check under certain conditions. Under such conditions, if no more *Ribes* become established, a peak presumably might be reached in certain localities. Even at Kelm Mountain, however, new infections are taking place each favorable season.

The peak of the damage cannot be said to have been attained, however, when only 11 per cent of the trees studied are now dead and 19 per cent of the doomed trees are still alive (13). The damage from infections on the trees at the present time has not matured yet, and there will surely be more infection of the pine. At present, no striking amount of damage is visible in the Adirondacks: there are no large numbers of dead trees, no large trees dead. The Kelm Mountain lot, in which 90 per cent of the trees are infected and at least 87 per cent doomed, still looks like a fine, healthy stand of 18-year-old pine.

In the Adirondacks, the second and third critical points have been reached. Because of the scarcity of *Ribes* or absence of favorable infection conditions in certain sections, the pine will not be generally destroyed, and may not be damaged at all. But where there are *Ribes* present under normal conditions, the maximum damage has not been done. The Kelm Mountain area now shows what may confidently be predicted where *Ribes* occur, and the Horicon lot shows what may surely be expected. The possible damage is not yet complete at either place, although it is imminent, inevitable, and prognosticable.

The data available lead us to believe that, with the momentum gathered since 1919, the next severe blister-rust attacks will be much more formidable. As this is being written, the reports seem to indicate that the epidemics of 1925 and 1927 were of sufficient magnitude to bear out this statement. The concept of blister-rust momentum, the progressive and continuous gathering of increasing force through the years—which in this particular case means ultimately the volume of sporidia—has never been completely, thoroughly, and adequately grasped.

SQUIRREL INJURY TO CANKERS

After particularly severe winters, it has been reported by several observers (14) that many cankers are almost completely gnawed away, presumably by squirrels and perhaps by porcupines and other rodents. Notes were taken in the first place merely to satisfy a curiosity as to how large a percentage of the cankers was destroyed by animals. It is certain that in some seasons they consume a great deal of aecial tissue. They seldom entirely clean off the diseased area, but usually consume all the living affected bark except a narrow zone of about enough tissue to continue growth the next spring. These animals must cause considerable diminution in potential production of aeciospores, for they consume nearly all the portion of the diseased bark which would produce aecia the next spring. Of 11,100 cankers examined, 4,600 or 41 per cent had been gnawed by rodents, and in most cases the injury was complete enough to prevent the formation of aecia the following spring.

The animals eat the bark of cankers of all sizes of branches and trunks, from very small ones up to those as large as 13 in. in diameter (Fig. 1) according to the writer's observations. In many cases, it is noted that rodents have eaten the new growth of the same cankers each year. In 1925 a new type of injury was noted, namely, the cutting off of small cankered twigs entirely. The stub ends of the twigs bear rust-infected tissue. The explanation seems to be that the small twigs will not support the weight of the animal while it eats the bark, and therefore the twig is snipped off and eaten where it is more convenient.

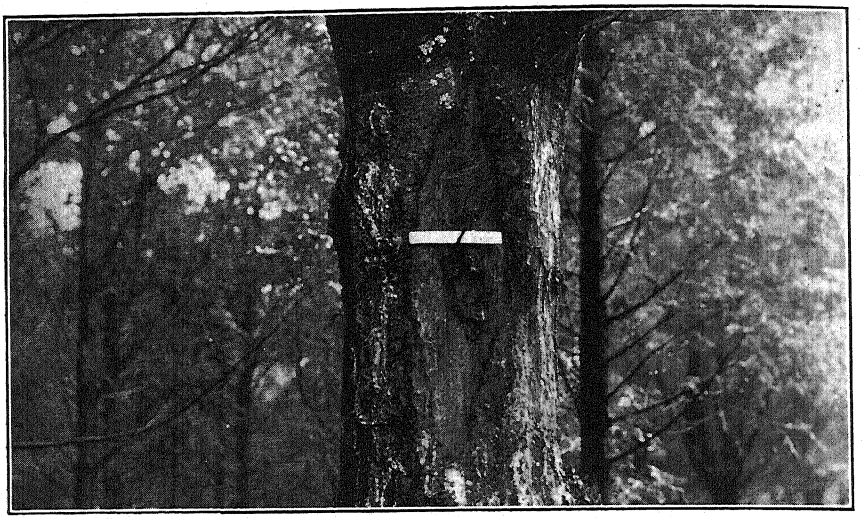


FIG. 1. Blister-rust canker on white pine gnawed by rodents. Horicon Plot, Horicon, N. Y.

The canker is now 30 in. by 18 in. The tree is 15 in. D. B. H. and at the canker is 15¾ in. in diameter as seen in the photograph and only 11½ inches at right angles to the canker because of the flattening effect. The fungus entered the trunk on the adventitious shoot seen in the center of the canker. The decorticated portion of the canker was gnawed out by rodents in the winter of 1922–23 and the line of demarcation represents all but the narrow rim of the canker at that time. The ruler resting upon the adventitious shoot is 6 inches long.

This rodent injury was very common during the winters of 1922–1923 and 1923–1924 in the Adirondacks, but was almost entirely lacking during the three succeeding winters. Less of it has been noted in Essex and Clinton Counties than in Warren County.

INVASION OF BLISTER-RUST CANKERS BY SECONDARY FUNGI

The possible effect of secondary fungi in destroying blister-rust cankers or preventing fruiting has attracted the attention of several workers (1, p. 650; 14, p. 29). The data at hand show that 20 per cent of the cankers are invaded by secondary fungi. In the McPhillips No. 1 plot, 60 per cent of the cankers were infected with other fungi. In most cases the invasion is quite complete, and sporulation of the rust fungus is prevented if the canker is not already dead.

The precise biological significance of secondary fungi in cankers is unknown. Undoubtedly, many cankers are invaded where the aecia rupture the bark and expose the inner-bark cells. Colley has suggested the joint action of the rust fungus and the secondary fungus in completing the

girdling of the stem (1, p. 650). It has also been assumed by many of those interested that the significance of the secondary fungus rests in its prevention of fruiting by killing the living bark. It is true that in many cases cankers which have never borne aecia are found attacked by secondary fungi. These may have entered the canker before the bark was dead and aided in the killing, or they may have entered after the tissue was dead. The exact relations can be determined in very few cases, but it appears that either of these courses is possible.

The fungi most commonly found on cankers are a species of *Phoma* (imperfect stage of *Caliciopsis pinea*) and *Cenangium abietis* (Pers.) Rehm. An unidentified species of *Stereum* (perhaps *S. sanguinolentum* Alb. & Schw.) has also been found occasionally. In 1927 a new fungus was found to be very common in the Dannemora Plantation, both as a secondary fungus on blister rust cankers and to a less extent on bark not infected by *Cronartium ribicola*. It was identified[1] for the writer as *Dasyscypha agassizii* (B. & C.) Sacc. This is apparently the first time this fungus has been reported upon *Pinus strobus*.

MORTALITY OF BLISTER-RUST-INFECTED WHITE PINE IN RELATION TO AGE OF TREES AT TIME OF INFECTION

Data based upon the damage studies mentioned earlier (13) show that the older the pines are when they are infected, the less chance there is of their being killed. The discussion is resumed here for amplification and in order to present more data. The facts have excited a little criticism and skepticism, and while they probably are subject to variation under certain conditions, on the whole there is some meaning in them. The new data are incorporated with the old in figure 2.

Logic and certain facts support the thesis. The blister-rust fungus enters the tree for the most part through the needles of the branches, except in the earliest years. Most of the cankers on trees are originally branch cankers, and after the tree is five years of age this is true in over 98 per cent of the cases. The reason is obvious. The scattered, unprotected tops are exposed to drying, while the lower branches—bushier, more abundant, closer together, nearer the ground, and surrounded by other vegetation—are bathed by air of relative humidity high enough for infection, for much longer periods than the tops. Counts of over 15,000 cankers on from 12- to 20-year-old trees show that 98 per cent of them are less than 3 ft. from the ground, 95 per cent less than 2 ft. In other words, they are down in the thick lower foliage where the relative humidity sinks later in the day

[1] The fungus was first sent to Dr. E. A. Burt through an error, and later to Dr. F. J. Seaver. Both identified it as noted.

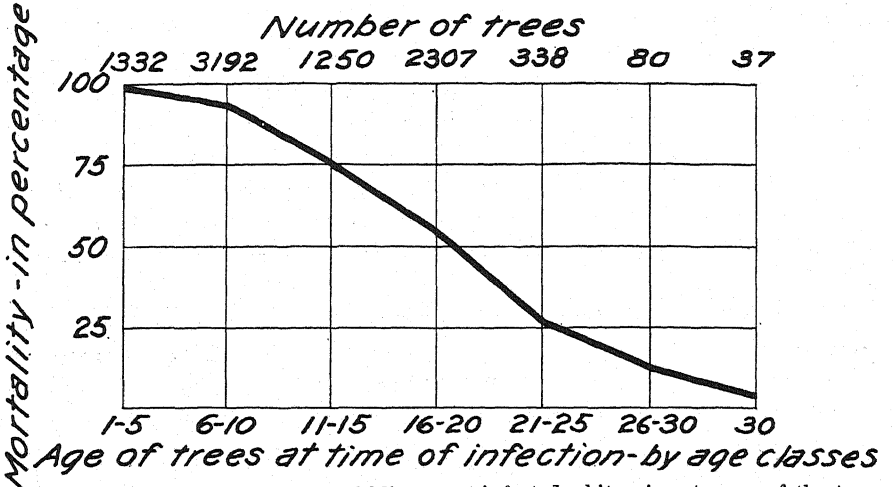


FIG. 2. Relation of the mortality of blister-rust-infected white pines to age of the trees at time of infection. Based on definite data from about 8,500 trees.

and rises earlier than up in the tops. Of the 2,134 cankers in the Dannemora Plantation and the 9,000 in the Kelm Mountain lot, only 1.1 per cent were in the open, unprotected by foliage.

Inasmuch, therefore, as the larger proportion of the cankers are branch cankers, and inasmuch as the needles on the branches persist only 3–5 years, it stands to reason that the difficulty encountered by the fungus in reaching the stem increases with the distance of the needles from the stem—that is, with the age of the tree, discounting the small number of top infections. In other words, the older the tree at the time it is infected, the greater the period of time consumed by the fungus in travelling from the place of infection on the branch to the stem where alone it can kill the tree; and the longer the fungus requires to reach the stem, the greater the opportunity for the tree to escape harm through the death of the fungus in the canker by shade, fungi, and insects or other animals. Point may be given to the foregoing statements by the following isolated facts: the recovery of 69 infected trees at Kelm Mountain since 1923 (meaning the death of cankers by shading so that the trees are no longer doomed to die); the dropping of the infection percentage at Kelm Mountain from 97 in 1923 to 90 in 1927, and the death of over half the 9,000 cankers in the lot by shading; the dead condition of 66 per cent of the cankers on the Whalen lot worked in 1925; and the 45 per cent of dead cankers on all the lots in the first studies with no accounting of conditions since.

The new data collected each year have substantiated the original results, with only slight changes in the mortality percentages for the different age classes.

RELATION OF PERCENTAGE OF INFECTION TO STOCKING OF STAND

Posey and Ford (10), in their paper on the infestation at Kittery Point, Maine, presented a curve showing that the degree of infection varies inversely as the stocking, apparently because of screening or some such cause, and in spite of the occurrence of moisture conditions more favorable for infection in the thicker stands. This general fact seems to hold true under certain conditions, especially where the infection is not heavy, but it is true in very few cases where there is sufficient infection of pine to provide a fair test.

When the infection percentages of the New York one- and two-acre plots were plotted in this way, the curve was a duplicate of the one given by Posey and Ford. But, on the other hand, in many of the plots with the heavily and the lightly stocked portions sharply separated, it was found that this relationship did not hold true except in a few cases. For example, at Kelm Mountain, with 97 per cent infection on the two acres, there was no difference in infection percentage whether the stocking was 3,000 to the acre or only 400. At Horicon, plots varying from ¼ to 1 acre in size with stockings of 600, 1,300, and 2,400 trees have 43 per cent infection, 41 per cent, and 80 per cent respectively. On the Whalen lot, a plot with 800 trees an acre had 63 per cent infection, while a plot with 2,500 trees an acre had 54 per cent. On the Sternberg lot, a plot with 4,800 trees an acre had 7 per cent infection and a plot with 8,960 an acre had 66 per cent infection.

All pertinent data obtainable in New York from the writer's work are represented in figure 3. It will be noted that, up to 3,500 trees an acre, the density of the stand apparently has no effect upon the degree of infection in the experimental plots. The plots with 4,000 or 5,000 trees an acre have a low percentage of infection, but a small plot, on the other hand, with 9,000 trees an acre (Sternberg B) has a high percentage. The Remington plots with 15,000 trees an acre have a low percentage of infection, but it is to be noted that the *Ribes* on this area are few in number and small in size. Furthermore, the pines have been exposed to infection in none of the important blister-rust years except 1925, and the damage resulting from infection in that year is not yet evident, if any great amount of damage could occur with so few *Ribes* as the source of infection.

One major criticism of attaching too much importance to this curve as representing a blister-rust generalization is that the data in any particular case are not complete. For example, in one of the Remington (Chestertown) centers in the graph, with 14,000 trees an acre, the infection was 6.4 per cent in 1925 and 15 per cent in 1927. With a large amount of *Ribes* instead of one or two scraggly bushes, this percentage would undoubtedly be higher even now. Too little is known of what happened to the

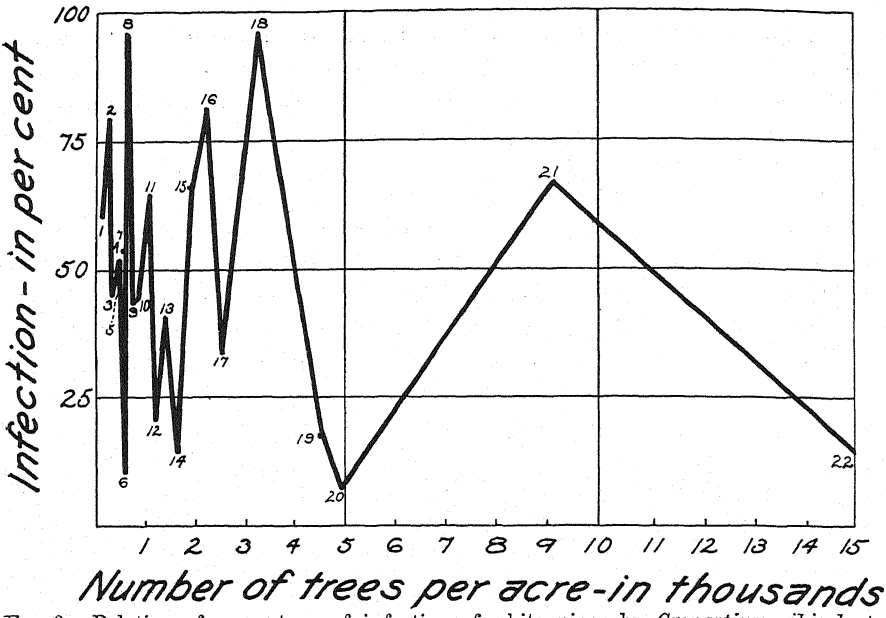


FIG. 3. Relation of percentage of infection of white pines by *Cronartium ribicola* to stocking of stand.

The numbered points on the graph represent the percentages pertaining to the following plots: 1—Myers; 2—Harkness; 3—American Steele and Wire Co.; 4—Goff; 5—Brown (the point ought to be nearer the 50 line than can be shown here); 6—McPhillips No. 2; 7—Downes; 8—Kelm Mt.; 9—Horicon No. 1; 10—Ames; 11—Whalen; 12—Smith; 13—Horicon No. 2; 14—Burdick; 15—McPhillips No. 1 (1924); 16—Horicon A; 17—McPhillips No. 1 (1926); 18—Kelm Mt. A; 19—Sternberg A; 20—Sternberg; 21—Sternberg B; 22—Remington.

reproduction on many of our New York plots just following the great 1919 blast. It is known that the Horicon plot had a large quantity of young reproduction in 1920, which was not there when the plot was studied in 1923. Our observations of many other lots, including Kelm Mountain plot, lead us to believe that similar conditions existed on those. The two-storied condition[2] of most of the New York plots show that our figures of stocking of stands represent only present conditions. The writer can never be convinced that any high degree of stocking would have kept the percentage of infection very low on the Kelm Mountain, Horicon, Whalen, Downes, Harkness, and McPhillips lots (13). It seems reasonable to assume that

[2] By "two-storied condition" is meant the presence upon the lots of trees under 10 years of age and over 17 years of age, without any between these limits. The younger class of trees came in after 1919 and the older class prior to 1911, while the series of great waves of infection from 1911 to 1919 inclusive destroyed most of the seedlings which started during this period. (See 13.)

a large percentage of the countless seedlings lost long before any studies were made were destroyed by blister-rust, and that if knowledge of these conditions were at hand the percentages of infection for these lots would be much higher than is shown by the record.

DEAD CANKERS

As a result of counts made of the number of dead cankers, it was found that 45 per cent of all cankers on the plots, on both dead and living trees, are now dead. They are dead either from injury by rodents, invasion by secondary fungi, killing of the bark by the blister-rust fungus itself, or shading out. On the Whalen lot, 57 per cent of the 1,500 cankers were dead at the time of study in 1925.

INSECTS, RODENTS, SLUGS, AND SNAILS FEEDING UPON PYCNIAL DROPS

The pycnial drops and pycnial tissues of blister rust cankers are sweet. The sugar present at the time of examination was found by Dr. Nathaniel O. Howard of the Office of Forest Pathology, U. S. Department of Agriculture, to be glucose. It has been assumed that squirrels and other animals eat the resinous pine bark partly because of its succulence, when other food is unavailable on account of snow. On the other hand, porcupines will eat extensive areas of healthy pine bark in some winters, without apparent regard for the resin. A recent paper by Hosley (4) reports that, in certain localities when the snow is deep, red squirrels have been found eating the buds of several coniferous trees, including white pine, and that they clip off the terminal shoots of white pines. Although it is not made clear in the paper, the squirrels apparently remove the terminal shoots of the white pine for the buds and not for the bark. It appears to be the succulence of the bud tissues that appeals to the squirrels, in spite of the resin, although it is not known whether the buds are sweeter than the normal bark tissue.

But whatever may be the attractive element in the pycnial zone of the bark, rodents have developed a liking for the sweet pycnial fluid. No squirrels have been observed feeding upon the pycnial drops themselves, but paired, chisel-like teeth-marks show that they scrape off the pycnia before the fluid has been exuded. In 1926 and 1927 it was found that the entire crop of pycnia in certain localities had been consumed.

It was not until 1924, however, that the writer noticed that the pycnial drops attracted insects in any considerable number. In 1924 and 1925 the pycnial drops were large and abundant because of the moistness of the seasons. Various hymenopterous insects such as ants, bees, and hornets were commonly observed feeding on the drops as long as they persisted, and also certain Hemiptera, Coleoptera, and Lepidoptera. The insects industriously and thoroughly cleaned up the pycnial areas, drop by drop.

Slugs and small snails also have been observed feeding upon the pycnial drops, especially in moist seasons such as 1924 and 1925.

SLUGS AND SNAILS FEEDING UPON AECIOSPORES

Different kinds of insects have been found upon the aecia of *Cronartium ribicola,* apparently in search of food. Insects and other animals have been found carrying aeciospores on their bodies (2, 3, 10, 14). It was not until 1927, however, that the writer observed animals eating aeciospores. In 1927 at Dannemora, early in the morning, or all day during wet weather, slugs and small snails were found feeding upon the aeciospores all through the season of aeciospore production, which lasted until July 17. They were seen with their mouth parts in the aecia like pigs at a trough, and when they were removed to a little distance they invariably returned to that position forthwith. Furthermore, aeciospores were found in the alimentary tracts of slugs captured on the aecia, and the feces of these slugs showed remains of aeciospore walls (See 3).

SUMMARY

1. This paper presents observations made in connection with studies on the damage caused by white pine blister rust, carried on in the Adirondacks for the New York Conservation Department.

2. The functions of the different spore stages of the blister-rust fungus are discussed from a biological point of view.

3. In the Adirondacks, between 60 per cent and 70 per cent of the cankers initiated prior to 1925 were on 1919 wood. The epidemic of 1925 appears to have been next in severity to that of 1919, if not more severe. The season of 1927 likewise appeared to be favorable for heavy pine infection.

4. In the life history of blister-rust there are four critical points. During the later phases, biological control may operate. In the Adirondacks, however, on account of the epidemics of 1925 and 1927, blister rust undoubtedly has not reached its peak.

5. During years unfavorable for blister rust, momentum is acquired for the voluminous showering of sporidia on the pines when conditions are favorable. The importance of this momentum has not heretofore been appreciated.

6. A certain amount of inoculum is destroyed by small animals that eat the cankers and by attacks of secondary fungi. *Dasyscypha agassizii* (B. and C.) Sacc. is reported on blister-rust cankers and on *Pinus strobus* for the first time.

7. Mortality of infected pine trees decreases with the age of the trees at the time of infection.

8. While the percentage of infection appears, in general, to vary inversely as the stocking of the stand, this tendency is apparent only because of unfavorable infection conditions in a small lot of young trees with 15,000 to an acre.

9. Forty-five per cent of the cankers on the experimental plots are now dead. On one lot, 57 per cent were dead in 1925.

10. Rodents, slugs, snails, and many insects feed on pycnia and pycnial drops.

11. Slugs and snails feed on the aeciospores.

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BORDEAUX SPRAY IN THE CONTROL OF FIRE BLIGHT OF APPLE[1]

MONROE MCCOWN[2]

Owing to the destructiveness in Indiana orchards of fire blight, caused by *Bacillus amylovorus,* and the fact that beneficial results from the use of a bordeaux spray have been observed by investigators and midwest orchard men, spraying tests and laboratory studies have been carried out at Lafayette and in Indiana orchards. The results of the field tests show that a comparatively weak bordeaux spray in the full-bloom period reduced the amount of blossom infection in Grimes and Jonathan apples. In the laboratory, bordeaux mixture and copper dusts apparently killed the bacteria. It is the purpose of this paper to present more in detail the evidence upon which was based a report[3] recently made to the Indiana fruit growers.

THE EFFECT OF A FULL-BLOOM BORDEAUX SPRAY UPON BLOSSOM INFECTION

On April 28, 1927, 100 clusters of blossoms on a Grimes tree in full bloom in the University orchard at Lafayette were sprayed once by means of an atomizer with bordeaux 1–3–50 (1 lb. $CuSO_4$, 3 lbs. hydrated lime, 50 gals. of water). Immediately after the bordeaux application, 50 of these clusters were inoculated by means of an atomizer containing a cloudy suspension of the fire-blight bacteria in distilled water. Of the remaining 50 clusters, 25 were similarly inoculated 24 hours later (April 29), and the remaining 25 inoculated 48 hours after the bordeaux was applied (April 30). Each day 25 unsprayed blossom clusters were inoculated as checks.

The inoculations were made between 1:30 and 3:00 P. M. The maximum temperatures as reported by the U. S. Weather Bureau for April 28, 29, and 30 were 69°, 68°, and 61° F., respectively. Following

[1] Contribution from the Department of Horticulture, Purdue University Agricultural Experiment Station, Lafayette, Indiana. Part of the data in this paper was presented to the Faculty of Purdue University, May, 1928, as a thesis in partial fulfillment of the requirements for the degree of Master of Science in Agriculture.

[2] The writer is indebted to Dr. Max W. Gardner, of the Department of Botany, Purdue University Agricultural Experiment Station, for laboratory methods and valuable advice and suggestions during the progress of the work; to Professor F. P. Cullinan, of the Department of Horticulture, and Professor P. H. Brewer, of the Department of Agronomy, for suggestions and advice; and to the Department of Botany, Agricultural Experiment Station, for laboratory facilities.

[3] MCCOWN, MONROE. Spraying for the control of fire blight in the apple. Trans. Ind. Hort. Soc. 67: 129–133. 1928.

the spray application, and prior to the inoculations of April 29, 0.36 in. of rainfall was recorded. Those clusters inoculated April 30 were subjected to an additional 0.32 in. of rainfall prior to inoculation. On April 28, when the spray was applied, there was an average of one unopened blossom in each cluster. The significance of this unprotected blossom will be shown later.

The clusters were watched closely and all infected blossoms were removed at intervals of two to three days and examined under the microscope to verify the diagnosis. The bacteria ooze out in conspicuous masses from the cut pedicels of blighted blossoms which are sectioned and mounted in water under the microscope. Two types of blossom infection were recognized: primary, in which the bacteria had entered presumably through the nectary; and secondary, in which the bacteria had entered the pedicel from the spur.

TABLE 1.—*Effect of bordeaux mixture 1–3–50 applied as a full-bloom spray upon the number of fire-blight infections in Grimes apple blossoms*

Time of inoculation, 1927,	Spray treatment	No. blossom clusters	Av. no. primarily-infected blossoms a cluster	Percentage of clusters infected
April 28, immediately after spraying	Bordeaux 1–3–50[a]	48	0.08	8
	None (Check)	24	1.46	87
April 29, 24 hours after spraying	Bordeaux 1–3–50	24	1.08	71
	None (Check)	24	2.24	96
April 30, 48 hours after spraying	Bordeaux 1–3–50	24	1.25	75
	None (Check)	25	1.88	88

[a] Bordeaux mixture made up at the rate of 1 lb. $CuSO_4$ and 3 lbs. hydrated lime to 50 gals. water.

The results, recorded in table 1, show that bordeaux 1–3–50 was very effective against blossom infection when the clusters were inoculated immediately following the bordeaux application. In this case, only 8 per cent of the sprayed clusters developed infection, while 87 per cent of the unsprayed check clusters became infected. Those clusters, inoculated 24 hours following the spray application, and after a rainfall of 0.36 in.,

developed considerably fewer infections than the checks. Only 71 per cent of the sprayed clusters developed infection, while 96 per cent of the check clusters blighted. While 75 per cent of those clusters inoculated 48 hours after spraying blighted, 88 per cent of the check clusters inoculated at the same time were infected. These clusters had been subjected to an additional rainfall of 0.32 in. prior to inoculation. No natural infections occurred in this tree.

A comparison of the number of primarily infected flowers in the sprayed and unsprayed clusters shows an even more striking difference than do the relative percentages of clusters infected. However, from a practical standpoint this means little, for, if one flower in a cluster develops primary infection, in all probability the remaining flowers in that cluster will be killed by secondary infection. Apparently the bacteria travel down the pedicel of the primarily infected blossom into the spur and up through the stems of the remaining flowers.

Upon first thought one might be inclined to attribute the poorer control obtained in those clusters inoculated 24 and 48 hours after spraying entirely to washing away and dilution of the bordeaux in the blossoms by the intervening rains. Because of the marked reduction in primary infection in these clusters, it would seem, however, that the one unprotected blossom in each cluster might account for much of this increased infection. Previous mention was made of the fact that at the time of spraying there was an average of one unopened blossom in each cluster. These blossoms opened between the time of spraying and the time of the two later inoculations and contained none of the protective spray material.

Spraying tests[4] were carried out in a number of Indiana orchards in 1928, a season in which fire blight was particularly prevalent.

On May 2, 1928, ten mature Jonathan trees, in the Experiment Station orchard located near Bedford, were sprayed once, when in full blossom, with bordeaux 1–3–50. Counts made on June 16 of the number of infected blossom clusters on each of the ten sprayed trees and seven unsprayed check trees showed a reduction of 79 per cent due to spraying. There was an average of only 68 blighted blossom clusters a tree on the sprayed trees, while the unsprayed-check trees averaged 329 infected clusters a tree. The number of infected clusters on the sprayed trees ranged from 4 to 187, and on the unsprayed checks, 171 to 938.

[4] The writer wishes to acknowledge the cooperation of Dr. B. A. Porter, U. S. Bureau of Entomology, Vincennes, Indiana, who applied the spray in the Vincennes test in connection with some of his own investigations; and of Mr. Hobert Hall, Superintendent of the Moses Fell Annex Farm, Bedford, Indiana, who made the full-bloom application in that orchard.

A similar test was made on Jonathan trees in an orchard near Vincennes. A count made of the number of blighted clusters a tree on June 5, on four representative sprayed trees and three check trees, showed a reduction of 52 per cent due to the one bordeaux application. The four sprayed trees averaged 209 infected blossom clusters a tree, while the three check trees contained an average of 440 blighted clusters each. The spray, bordeaux 1–3–50, was applied May 1, when the trees were just coming into full blossom.

In a small test, involving three sprayed pear trees and one unsprayed tree in an orchard near Lafayette, the number of infected blossom clusters a tree was reduced 98 per cent. The three sprayed trees contained an average of only 10 infected clusters a tree, while there were 474 blighted clusters in the check tree when the counts were made on June 2. The one spray application of bordeaux 1–3–50 was made May 3, when approximately 80 per cent of the blossoms were open. In all of these tests, the only departure from the recommended spray schedule was the addition of the one full-bloom spray. No bordeaux was used in any of the other early applications.

These results, showing that bordeaux reduces blossom infection, are in accord with the findings of McCue[5] in Delaware, who, in 1907, sprayed five Bartlett pear trees with bordeaux when in full blossom with a consequent reduction in the number of infections. Stevens, Ruth, Peltier, and Malloch,[6] in Illinois, reported complete control of fire blight infection in two Kieffer pear trees by spraying with bordeaux through the period of blossoming and for some time thereafter. Although the conditions are not exactly comparable, it is of interest to note that Reimer,[7] who worked with five varieties of pears in Oregon, concluded after three years' investigation that bordeaux would materially reduce the number of blight infections.

No russetting of the fruit occurred in any of the Indiana tests mentioned above. The same strength of bordeaux has been used as a full-bloom spray in other orchards in Indiana during the past three years and in no case has serious russetting resulted. Furthermore, no reduction in set of fruit has been observed in any of the tests. No arsenic was used in any of the tests.

LABORATORY TESTS

Weak Alkaline Bordeaux

Laboratory studies were begun in 1926 to determine the effect of bordeaux in various forms upon the fire-blight bacteria. Bordeaux was made

[5] MCCUE, C. A. Pear blight. Trans. Peninsula Hort. Soc. **1917**: 51–55. 1917.

[6] STEVENS, F. L., W. A. RUTH, G. L. PELTIER, and J. R. MALLOCH. Observations on pear blight in Illinois. Trans. Ill. State Hort. Soc. **50** (1916): 216–227. 1917.

[7] REIMER, F. C. Value of bordeaux mixture in blight control. Trans. Ore. State Hort. Soc. **17** (1925): 136–142. 1926.

up from a stock solution of $CuSO_4$ and a stock suspension of hydrated lime. In some cases these stock preparations were sterilized by heating in an autoclave, but this procedure was found to be unnecessary. In each of a series of test tubes, 3 cc. of bordeaux were made up at such a strength that the addition, to each tube, of 1 cc. of a cloudy suspension of the fire-blight bacteria (from pure culture of proved pathogenicity) in sterilized distilled water just brought the bordeaux up to the strength desired. At specified time intervals, following the introduction of the bacteria, loop inoculations were made from the bordeaux tubes into tubes of melted potato agar or beef-dextrose agar and plates were poured. For a control, one or more plates were poured from tubes of agar inoculated with a loopful of the bacterial suspension diluted one to three with sterilized distilled water. The colonies that developed in these plates were counted.

The results of three series of laboratory tests with bordeaux, of strengths varying from 1/4–3/4–50 to 1/64–3/64–50, are presented in table 2. These

TABLE 2.—*Effect of liquid bordeaux upon the fire-blight bacteria when the bacteria are suspended in the mixture and exposed to its action for varying periods*

Series	Suspension medium	No. colonies produced on poured agar plates after exposures indicated				
		2 min.	4 min.	6 min.	12 min.	24 min.
	Water (Check)	.....	.....	.....	.....	12,000
A	Bordeaux 1/4–3/4–50[a]	960	.....	0	0	0
	do 1/8–3/8–50	9,500	.....	44	0	0
	do 1/16–3/16–50	10,000	.....	194	0	0
	Water (Check)	.....	.....	.....	.....	15,000
B	Bordeaux 1/16–3/16–50	13,000	.....	0	0	0
	do 1/32–3/32–50	12,000	.....	5	0	0
	do 1/64–3/64–50	14,000	.....	1	0	0
	Water (Check)	.....	.....	.....	.....	4,500
C	Bordeaux 1/8–3/8–50	162	43	0	0	0
	do 1/16–3/16–50	37	2	0	2	0

[a] Bordeaux mixture made up at the rate of ¼ lb. $CuSO_4$ and ¾ lb. hydrated lime to 50 gals. water.

results show that bordeaux in the liquid state apparently killed the bacteria after comparatively short exposures. Very few colonies were produced in those plates poured after an exposure of 6 minutes and in no case were colonies found in those poured after an exposure of 24 minutes. It is also

interesting to note that the weaker mixtures of bordeaux were apparently as effective as the stronger ones.

Dilute Lime Water

It was known that the bordeaux used in the above tests was highly alkaline. The effect of lime water upon the bacteria was next tested in an effort to determine whether the apparent killing might be due to the action of the hydroxyl ion.

To make up lime water that would be fairly comparable to the concentration of lime in a 1/4–3/4–50 bordeaux, hydrated lime was added to water at the rate of ½ lb. to 37½ gals. of water. One cc. of a cloudy suspension of the fire blight bacteria in sterilized water was then added to three cc. of the lime water. The resulting strength of the lime water was equivalent to ½ lb. of hydrated lime to 50 gals. of water. Plates were poured as in the tests with alkaline bordeaux previously described, and the colonies that developed in the plates were counted. The results of two series of tests with lime water are recorded in table 3.

TABLE 3.—*Effect of lime water upon the fire-blight bacteria when the bacteria are suspended in the solution and exposed to its action for varying periods*

Series	Suspension medium	No. colonies produced on poured agar plates after exposures indicated						
		2 min.	6 min.	12 min.	24 min.	30 min.	45 min.	50 min.
	Water (average of 3 plates)	.....	.....	.....	.....	.....	.....	15,000
A	Lime water (½ lb. to 50 gals. water	16,000	2,000	1,200	11,000	207	298	142
B	do	5,330	3,300	3,150	145	289	7	0

While the lime water apparently killed or inactivated the bacteria, the action was much slower than that of the liquid bordeaux. This slower action of the lime water suggested the presence of some toxic factor other than the hydroxyl ion in the liquid bordeaux.

Neutral Bordeaux

Neutral bordeaux was made up by adding lime water to a solution of copper sulphate until the resulting suspension showed a pH of approximately 7.2 when tested with brom thymol blue. The bordeaux was tested with potassium ferro-cyanide and no free copper found to be present. Only

enough lime was used to neutralize the copper sulphate and insure the absence of any free copper. The method of testing the effect of this bordeaux upon the bacteria was the same as that used for the alkaline bordeaux. One cc. of a suspension of the bacteria in sterilized water was added to three cc. of the bordeaux, which then contained the equivalent of ¼ lb. of $CuSO_4$ to 50 gals. of water, and plates were poured at definite time intervals. Five series of plates were poured. In no case were colonies produced after an exposure of 45 minutes, and in most cases no living bacteria were found after an exposure of 24 minutes to the bordeaux. The checks showed normal growth. Neutral bordeaux, therefore, was more toxic than lime water, though not quite so toxic as the alkaline bordeaux. These results indicate that the apparent killing power of the liquid bordeaux is not all due to the action of the hydroxyl ion. The action of this ion alone, at a concentration of pH 7.2, could scarcely account for the toxicity shown in these tests.

Aluminium Hydroxide

If the copper ion is inactive in bordeaux as long as there is an excess of lime present, the question arises as to whether the colloidal films in the bordeaux might not inactivate the bacteria by adsorption. To test this hypothesis, aluminium was substituted for copper in the bordeaux formula.

Lime water was added to a solution of aluminium sulphate until the pH of the supernatant liquid was approximately 7.0. The suspension was made up in such a strength that after the addition of 1 cc. of a suspension of the fire-blight bacteria in sterilized water to 3 cc. of the aluminium hydroxide, it contained the equivalent of ¼ lb. of the aluminium sulphate to 50 gals. of water. This material was tested in the same manner as the bordeaux in the previous tests.

No reduction in the number of the bacterial colonies produced on poured-agar plates occurred in the three series of tests after exposures ranging from 2 to 60 minutes. This indicated that no killing or inactivation could be attributed to adsorption by the colloidal films. These results, together with the fact that neutral bordeaux was effective, indicate that the copper itself may possibly kill the bacteria even though the copper is present in a colloidal state.

Dried Bordeaux Films

Known strengths of bordeaux were made up and, by means of a pipette, 0.3 cc. was placed upon each of eight microscope slides in sterilized petri dishes. The drops were allowed to dry for 24 hours. The covers were partially lifted from some of the dishes in order to expose the drop to the air in the laboratory during the drying period. The other dishes were left covered and the drops on these slides dried much more slowly.

At the end of the 24-hour period, 0.3 cc. of a cloudy suspension of the fire-blight bacteria in distilled water was placed on the area occupied by the dried film on each slide. From each of these drops, one loopful was plated out at specified time intervals. Similar loopfuls of the original suspension were plated as checks.

The results of two series of tests, as recorded in table 4, show that all

TABLE 4.—*Effect of dried bordeaux films upon the fire-blight bacteria*

Spray material on slide	Method of drying	No. colonies produced on poured agar plates after exposures indicated				
		5 min.	10 min.	20 min.	40 min.	60 min.
None (check)	.....	.....	.....	.....	.....	30,000
Bordeaux 1/16–3/16–50[a]	Covered	3,500	740	0	0	0
do	Exposed	18,000	15,000	540	0	0
Bordeaux 1/4–3/4–50	Covered	20,000	1,600	0	0	0
do	Exposed	24,000	18,000	3,800	0	0
None (check)	.....	.....	.....	.....	.....	33,000
Bordeaux 1/16–3/16–50	Covered	17,000	900	0	0	0
do	Exposed	27,000	25,000	6,000	133	0
Bordeaux 1/4–3/4–50	Covered	21,000	500	0	0	0
do	Exposed	26,000	21,000	4,000	0	0

[a] Bordeaux mixture made up at the rate of 1/16 lb. copper sulphate and 3/16 lb. hydrated lime to 50 gals. water.

bacteria were killed after a one-hour exposure to the films and after a 20-minute exposure to all the films dried slowly in the covered dishes. It is interesting to note that the bordeaux which was allowed to dry inclosed in the covered petri dishes was more effective than that which dried exposed to the air in the room, and closely approximated in toxicity the alkaline bordeaux tested in the liquid state. When the drops of bacterial suspension were placed upon the dried films, it was noted that those films which had dried in the closed dishes became re-suspended in a form closely resembling the freshly prepared bordeaux, whereas, those which dried exposed to the air in the laboratory remained attached to the slides.

Copper Dusts

Certain fungicidal dusts were tested by dusting flamed microscope slides by means of small insect-powder guns. The slides, while held in a vertical position, were tapped gently to remove any excess of dust and then placed in petri-dish damp chambers. A large drop of a cloudy suspension of the bacteria in sterilized water was placed on each dusted slide. Plates

were poured at definite time intervals from tubes of agar inoculated with a loopful from the drops on the slides. Similar loopfuls from the original bacterial suspension were plated as checks.

The bacteria were killed after the following exposures to the various dusts: bordeaux dust, containing 11 per cent metallic copper, 40 minutes; colloidal copper-lime dust, containing 15 per cent metallic copper, 20 minutes; Holland's basic copper sulphate, diluted with an equal amount of hydrated lime, 40 minutes; Kiltone dust, containing 8 per cent metallic copper, 40 minutes; copper-lime dusts, containing 20 per cent, 25 per cent and 27 per cent dehydrated copper sulphate, 40–60 minutes. Possibly copper dusts would prove as effective as liquid bordeaux in the prevention of blossom infection.

SUMMARY

Inoculation tests showed that a weak bordeaux spray (1–3–50), applied in the full-bloom period, reduced fire-blight infection in Grimes clusters inoculated immediately after spraying. Only 8 per cent of the sprayed clusters were infected, while 87 per cent of the check clusters blighted.

Bordeaux 1–3–50 applied when the clusters were opening into full bloom reduced natural infection 79 per cent and 52 per cent, respectively, in two field tests with the Jonathan apple and 98 per cent in one test on pear.

In the laboratory, alkaline bordeaux in strengths varying from 1/4–3/4–50 to 1/64–3/64–50 killed the bacteria after short exposures.

A weak lime water (½ lb. to 50 gals. of water) killed the bacteria, though not so quickly as the bordeaux. This indicated that the toxicity of the alkaline bordeaux might be due, at least in part, to the action of the hydroxyl ion.

Neutral bordeaux (pH 7.2) was found to be almost as effective as the alkaline bordeaux against the organism in laboratory tests, a fact indicating that some toxic factor other than the hydroxyl ion was present.

The fact that alminium hydroxide did not kill the bacteria in similar tests indicated that the effectiveness of the bordeaux could not be attributed to adsorption of the bacteria by the colloidal films.

When drops of a bacterial suspension were placed on bordeaux films dried on slides, the organisms were promptly killed. It was found that those films that were dried while inclosed within petri dishes apparently became resuspended upon the addition of the bacterial suspension and were more effective than were the films dried while exposed to the air in the laboratory. Films of copper-lime dusts likewise killed the bacteria.

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VIABILITY OF CERTAIN PLANT PATHOGENES IN SOILS

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Comparatively little is known about the overwintering and longevity of plant-pathogenic bacteria in the soil. Much time and effort have been devoted to a study of the behavior of the organisms in the plant and in pure culture, but the biology of these organisms in the soil has been largely neglected. This is probably due to the difficulties encountered in isolating plant pathogenes from field soils. The medium devised and described by the author (11), utilizing crystal violet bile agar has, in a measure, overcome this obstacle in such studies of plant pathogenic bacteria.

In this paper it is proposed to describe the results obtained on overwintering of *Pseudomonas tumefaciens* Sm. and Town. and seven other plant pathogenic bacteria in sterilized and unsterilized soil kept in the laboratory and in the open.

The findings of other workers are briefly as follows. Rand and Enlows (14), while studying the longevity of *Bacillus tracheiphilus* Sm., showed that the organism did not overwinter in the soil. Anderson and Chapman (2), working with *Pseudomonas tabacum* Wolf and Foster, found that freezing of the pathogene on agar streaks did not affect its viability. They also showed that infested soil was one of the main sources of infection to a new crop. Their field experiments indicated that the wildfire organism was able to survive the winter in the soil. Later, Anderson (1) found that the agar cultures of the pathogene survived a very severe winter in New England. He also showed that the bacteria overwintered in sterilized soils in the open, but apparently not in unsterilized soil. Clinton and McCormick (5) also showed that the direct application of overwintered infested soils to tobacco plants produced typical infection in one out of several trials. While studying the same pathogene, Johnson and Murwin (7) found that it might live over winter in dry soil or in dry leaves, but not in moist soil nor in decaying leaves.

Fromme and Wingard (6), working with *Ps. angulatum* Fromme and Murray, found that the pathogene overwintered in the soil in the open in the state of Virginia.

[1] The writer wishes to express his sincere gratitude to Dr. I. E. Melhus and Dr. J. H. Muncie for their valuable suggestions and criticisms made during the entire period of this work. These studies have been carried out in connection with the joint crowngall project of the Crop Protection Institute of the National Research Council; United States Department of Agriculture, Office of Mycology and Disease Survey; Iowa State College; and University of Wisconsin.

Clayton (4) was unable to demonstrate that *Ps. campestris* (Pam.) Sm. lives over winter in the soil in New York state. Kendrick (8), while studying *Ps. holci* Kendrick, noted that the organism was virulent after 50 days' freezing in the refrigerator. His field observations suggested that the organism overwintered in the soil. Skoric (15) was unable to show the overwintering of *Ps. pisi* Sackett in field soils in the open.

McCulloch (9) obtained infections on healthy treated gladioli by planting the corms in soil where the disease caused by *Ps. marginatum* McC. had been prevalent for several years. Muncie (10) has shown that *Ps. phaseoli* Sm. overwintered in sterilized soil in Michigan.

Probably the writer was the first to isolate *Ps. tumefaciens* from a composite sample of infested field soil taken in a nursery. He (12, 13) also showed that the crown-gall pathogene overwintered in field soils, which fact has recently been confirmed by Banfield (3).

ISOLATIONS AND INOCULATION TRIALS WITH EIGHT BACTERIAL PLANT PATHOGENES

The author (13) has already shown that *Pseudomonas tumefaciens* may live 16 months in unsterilized soils. Later, further experiments were carried on to determine by means of isolations and inoculations the longevity of *Ps. tumefaciens,* when carried in unsterilized and sterilized soils of various types in the laboratory.

Two sets of soil samples placed in flasks, including sterilized and unsterilized clay, loam, and sand were infested on June 3, 1926, with a 72-hour broth culture of the crown-gall pathogene. One set of flasks was sealed with paraffin and kept at room temperature, while the other, unsealed, was kept in the refrigerator. The isolations were made from the unsealed set at frequent intervals, while the sealed set was opened for the first time after 480 days from the time of infestation. Since the soil was fairly dry in the sealed set, sterile distilled water was added. The method of isolation was the same as previously employed by the writer (11). The results of isolations and inoculations from both sets of soils made at irregular intervals are given in table 1.

It will be seen from table 1 that virulent crown-gall bacteria were recovered from sealed and unsealed samples of infested sterilized clay, loam, and sand 736 days after infestation. Tomato plants were infected when inoculated with organisms which were isolated from the unsterilized soils in sealed flasks. Isolations were made from clay after 539 days, and from loam and sand after 669 days. In five trials organisms from unsealed, unsterilized clay produced no infection, while those from loam and sand produced infection after 587 days. These data indicate that *Ps. tumefaciens* may live in a virulent state for a long time in sterilized and unsterilized

TABLE 1.—*Longevity of Pseudomonas tumefaciens in sterilized and unsterilized soils, infested on June 3, 1928, and kept in the laboratory, as determined by inoculations of tomato plants with suspected plate colonies*

Date of isolations	No. days organisms in the soil	Tomato plants infected[a] with suspected colonies from infested soils												
		Sealed						Unsealed[b]						
		Sterilized			Unsterilized			Sterilized			Unsterilized			Check
		Clay	Loam	Sand	Clay	Loam	Sand	Clay	Loam	Sand	Clay	Loam	Sand	
Sept. 24, 1927	480	+	+	+	−−	−−	−−	+	+	+	−	−	−	−
Nov. 21, 1927	539	+	+	+	+	−	+	+	+	+	−	−	−	−
Jan. 8, 1928	587	+	+	+	−	−	+	+	+	+	−	+	+	−
Feb. 27, 1928	637	−−	−−	+	−	−	−	+	−−	−−	−	−	−	−
Mar. 30, 1928	669	+	+	−−	−	+	+	+	+	+	−	−	−	−
Apr. 10, 1928	680	+	+	+	−	−	−	+	+	+	−	−	−	−
Apr. 27, 1928	697	+	+	+	−	−	−	+	+	+	−	−	−	−
May 3, 1928	703	+	+	+	−	−	−	+	+	+	−	−	−	−
May 5, 1928	705	+	+	+	−	−	−	+	+	−	−	−	−	−
June 5, 1928	736	+	+	+	−	−	−	+	+	+	−	−	−	−

[a] + = Galls produced.
−− = No isolations made.
− = No infections obtained.

[b] Previous studies were reported by the writer (13, p. 335, table 1).

soils. The organism lives longer in unsterilized sand than in clay or loam.

The fact that the crown-gall pathogene overwinters in the soil suggested the advisability of determining whether other plant pathogenes could overwinter in the soil. In these studies, cultures of eight bacterial plant pathogenes were employed, namely, *Ps. tumefaciens, Ps. marginatum, Ps. phaseoli, Ps. beticolum* Sm., Brown and Town., *Bacillus carotovorus* Jones, *B. atrosepticus* van Hall, *Ps. tabacum,* and *Ps. citriputealis* C. O. Sm.

About 15 gms. of soil were distributed in ordinary culture tubes and sterilized at 15 pounds pressure for four hours. Dilution plates poured from these tubes remained sterile. In a similar manner, tubes of unsterilized soil were prepared. These sterilized and unsterilized soil samples were then infested by adding a sufficient amount of a 72-hour neutral beef-broth culture of the pathogenes to moisten the soils.

These cultures, covered with a piece of oiled paper to keep out water, were exposed in the following manner: the tubes of *Ps. tumefaciens, Ps. marginatum, Ps. phaseoli, Ps. beticolum, Bacillus carotovorus,* and *B. atrosepticus* were buried from 10 to 12 inches in the soil in the open at different times beginning October 25, 1927; while *Ps. tabacum* and *Ps. citriputealis* were placed on the surface of the soil in the open on January 12, 1928.

The temperature records between October 25, 1927, and March 10, 1928, were obtained from the official records of the U. S. Weather Bureau substation located at Ames, Iowa. The temperature during October was above freezing. The maximum temperature in November ranged from −5° to 20.5° C., the minimum for the same period being from −13° to 5.5° C. The lowest temperature occurred in December when it dropped to −32° C. on December 7. The minimum temperature for December ranged from −32° to 1.5° C., while the maximum ranged from −1.05° to 5° C. The minimum temperature in the month of January, 1928, ranged from −24.5° to 2° C., while the maximum during the same period was from −17° to 16.5° C. The range of minimum temperature in February was from −18° to 2° C., while the maximum was from −9.5° to 15.5° C. The maximum temperature for the first ten days of March ranged from −0.5° to 12° C., while the minimum ranged from −12.5° to 1° C.

Isolations could be made from only the sterilized soils carrying *Ps. tabacum, Ps. citriputealis,* and *Ps. beticolum* because these organisms were very sensitive to crystal violet bile medium. Isolations from the sterilized soil samples were made, and neutral potato-dextrose agar used. For the organisms other than *Ps. tumefaciens* the bile agar was slightly modified. The changes consisted in reduction of sodium taurocholate from 3 to 2 gms., and crystal violet from 2 to 1 cc. and an addition of 3 gms. of malt

extract. Only the bile agar was used for infested, unsterilized soil samples. The overwintered soil samples were brought into the greenhouse on March 10, 1928, and the dilution plates were made the next day. The pathogenicity of the cultures was proved by inoculating susceptible hosts with the suspected colonies. The results of these trials are presented in table 2.

TABLE 2.—*The pathogenicity of eight bacterial plant pathogenes taken from sterilized and unsterilized loam kept in the open during the winter of 1927–28, as determined by inoculations of susceptible hosts with suspected plate colonies*

Organisms	Condition of soil used for infestation	Date placed outdoors	No. days outdoors	Position of cultures	Pathogenicity of suspected plate colonies on susceptible hosts
Ps. tumefaciens	sterile	Oct. 31, 1927	132	12 in. below ground	+[a]
do	unsterile	do	do	do	+
Ps. phaseoli	sterile	do	do	do	+
do	unsterile	do	do	do	+
Ps. marginatum	sterile	Oct. 25, 1927	138	do	+
do	unsterile	do	do	do	+
B. carotovorus	sterile	do	do	do	+
do	unsterile	do	do	do	+
B. atrosepticus	sterile	do	do	do	+
do	unsterile	do	do	do	+
Ps. tabacum	sterile	Jan. 12, 1928	58	On surface	+
Ps. citriputealis	sterile	do	do	do	+
Ps. beticolum	sterile	Nov. 12, 1927	119	12 in. below ground	+

[a] + = Infections on susceptible hosts.

It will be seen from table 2 that *Ps. tumefaciens, Ps. marginatum, Ps. phaseoli, Bacillus carotovorus,* and *B. atrosepticus* lived over winter in sterilized and non-sterilized soils held in the open, while *Ps. tabacum, Ps. citriputealis,* and *Ps. beticolum* overwintered in sterilized soils. Whether the last three organisms can survive the winter in unsterilized soil is still a question. It should be said that the failure to recover the three last-named organisms may have been due to lack of a suitable selective medium.

SUMMARY

Virulent cultures of *Ps. tumefaciens* were isolated from sterilized infested clay, loam, and sand held in the laboratory for 736 days after

infestation. Infection on tomatoes was produced with colonies obtained from unsealed and sealed unsterilized loam and sand cultures 587 and 669 days, respectively, after infestation, and in the case of clay after 539 days.

Pseudomonas tumefaciens, Ps. marginatum, Ps. phaseoli, Bacillus carotovorus, and *B. atrosepticus* lived over winter in sterilized and non-sterilized loam soil held in the open. *Ps. tabacum, Ps. citriputealis,* and *Ps. beticolum* were recovered from overwintered, sterilized loam soil cultures.

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A COMPARATIVE STUDY OF SCLEROTINIA MINOR JAGGER AND SCLEROTINIA INTERMEDIA RAMSEY IN CULTURE

A. H. CHIVERS[1]

Smith (4) first reported in 1900 the occurrence of a small sclerotial fungus which was similar in some respects to *Sclerotinia libertiana* Fckl. and which he found destructive on lettuce in Massachusetts. The sclerotia produced by the fungus were decidedly smaller than those of *S. libertiana.* Smith was unable to obtain apothecia from the sclerotia of this form, but, since in one culture large sclerotia appeared among the small ones, he considered that the fungus was a degenerate form of *S. libertiana.*

Later the same fungus was isolated by other workers from lettuce in the vicinity of Boston and New York; at South Lima, in western New York; at Rochester, New York; Philadelphia, Pennsylvania; and Sanford, Florida. Jagger (1) made a study of the strains collected from these localities and concluded that they represented a species distinct from *S. libertiana,* to which he gave the name *Sclerotinia minor* n. sp. In his original description Jagger characterized the species, as far as the sclerotia are concerned, as follows: "Sclerotia black, irregular, 0.5–2 mm. in diameter, often anastomosing to form irregular, flattened bodies which are several millimeters in length." Since the appearance of the paper by Jagger on this species, the fungus has been found in many localities, including at least one station in Japan, and on several other suscepts besides lettuce.

Ramsey (2), in 1924, described a new species, *Sclerotinia intermedia,* which he isolated from decaying roots of salsify in 1920 and in the following year from decaying carrots. He distinguishes it from *S. libertiana* and *S. minor* chiefly by the intermediate size of its sclerotia at room temperature on standard potato, oatmeal, and bean agar; by differences in measurements of ascospores and asci; and on the basis of its lower temperature requirements for mycelial growth.

OBJECTIVES OF THIS INVESTIGATION

In Professor Whetzel's collection of cultures of species of *Sclerotinia* at Cornell University are various strains[2] which, in the light of present knowledge, he regards as *S. minor* Jagger. They have been isolated from various suscepts from widely separated localities. The writer has undertaken a study of these to determine, if possible, whether the variations in these

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[2] The term strain is here used in the sense of a culture obtained from a given collection of the fungus.

strains indicate merely those differences which the individuals of any species may be expected to show; whether they are differences of racial character within the species; or whether they are of such a nature and of such constancy as to justify the separation of certain of the forms into species distinct from *S. minor*.

Mr. B. Baribeau, District Inspector of the Dominion Field Laboratory at St. Anne de la Pocatière, Quebec, Canada, who isolated and sent cultures of two strains of *S. minor* to Professor Whetzel, called attention to the relatively small size of sclerotia of one of these (S451) when grown at a temperature of 10° C. as compared with sclerotia in cultures of the same strain grown at 18–20° C. The writer has undertaken to determine whether this is true for all the strains of *S. minor* listed below.

As Ramsey (3, p. 604) has pointed out the tendency of *S. intermedia* to form larger sclerotia at the lower temperatures, it was decided to include this species for comparative purposes in our temperature studies.

STRAINS STUDIED

In the following list of the several strains of *S. minor,* the first number is that under which the strain is listed in Professor Whetzel's personal catalogue of his stock cultures. The second number is that under which the specimen from which the strain was isolated is entered in the herbarium of the Department of Plant Pathology at Cornell University. The date is that of the year in which the strain was isolated.

S2, *15517,* on greenhouse lettuce, Irondequoit, New York, 1915.

S3, *16204,* on field grown lettuce, South Lima, New York, 1915.

S86, *15515,* on field grown lettuce, Long Island, New York, 1919.

S219, *15520,* on *Chrysanthemum cinerariaefolium,* Japan, 1924.

S334, *15518,* on sunflower, Ontario, Canada, 1925.

S451, *16205,* on potato, Quebec, Canada, 1927.

S452, *16206,* on sunflower, Quebec, Canada, 1925.

Cultures S2 and S3 are two of the original strains used by Jagger (1) in his investigations. The culture of *S. intermedia* Ramsey (S127) is the original strain on the bases of which Ramsey described the species. It is entered in the herbarium of the Department of Plant Pathology at Cornell University under the number *15508.*

Each of the forms named in the foregoing has been grown repeatedly in triplicate on potato dextrose agar made according to a standard formula (Whetzel 5, p. 230) in both petri dishes and 150-cc. or 200-cc. Erlenmeyer flasks, at temperatures[3] of 25°, 21°, 18°, 12°, 9°, and 6° C. The production

[3] Daily records of incubator temperatures, taken while the cultures were growing, showed that while some were constant, others varied somewhat in both directions from the figure given. In the opinion of the writer, however, these variations were not sufficient to influence materially the results obtained.

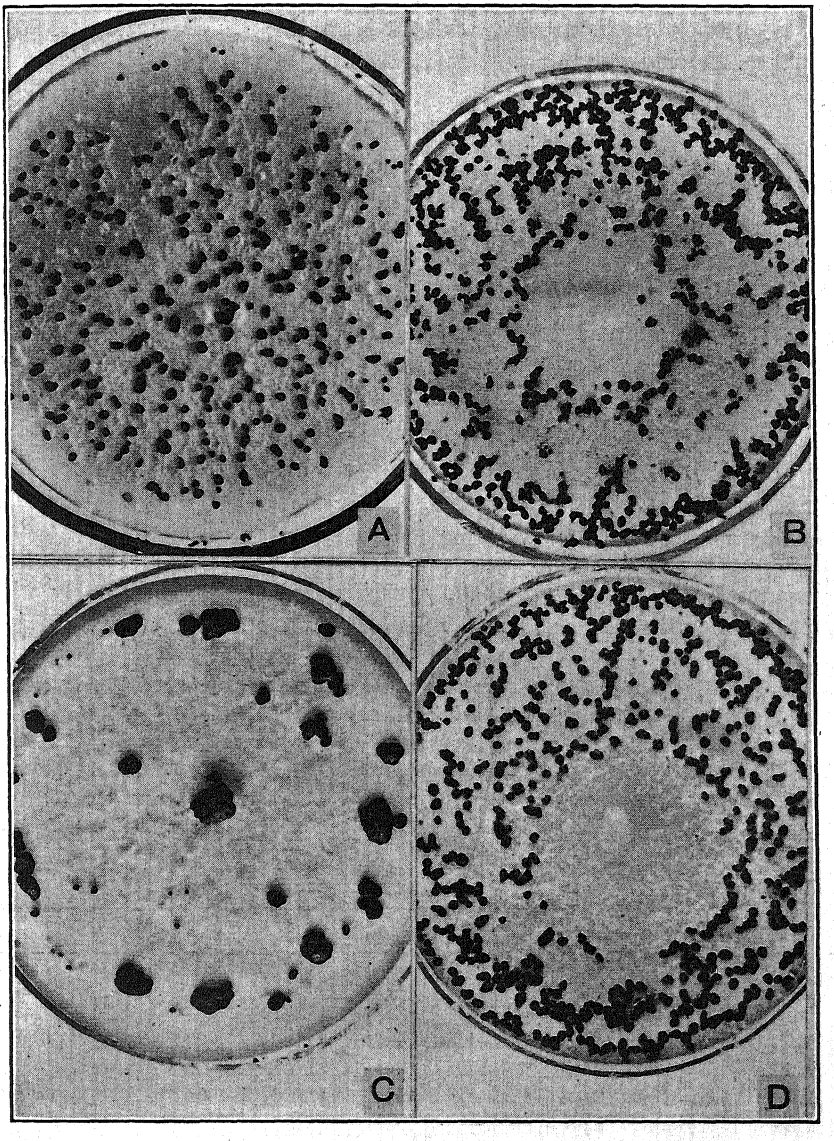


FIG. 1.—A and B. Cultures of *S. minor* (S3) showing the characteristics of race 1 when grown at 25° and 18° C. respectively.

C and D. Cultures of *S. minor* (S334) showing the characteristics of race 2 when grown at 25° and 18° C. respectively.

and characteristics of the sclerotia grown under these conditions have been carefully compared. All cultures photographed for this paper were grown on a layer of potato dextrose agar about 1 cm. thick in the bottom of 150-cc. or 200-cc. Erlenmeyer flasks.

RESULTS

Races of Sclerotinia minor

It was found that the strains of *S. minor* studied can invariably be separated into three groups which are designated as races.

Race I includes S3 and S219. At 25° C. (Fig. 1, A) the sclerotia are relatively large, black, oval or spherical, with little tendency to fuse, 0.25–2.0 mm., av. 1.25 mm. in diameter. The aerial mycelium is appressed, powdery, white, with substratum bare, especially near the margin. At 18° C. (Fig. 1, B) the sclerotia are smaller, black, with tendency to fuse, 0.25–1.5 mm., av. 0.80 mm. in diameter. The aerial mycelium is similar to that at 25° C.

Race II includes S2, S86, and S334. At 25° C. (Fig. 1, C) the sclerotia are formed in fused masses. These masses are relatively few, large, black, irregular in shape, 1.0–5.0 mm., av. 2.44 mm. in diameter. The aerial mycelium is abundant, appressed, powdery or felty, conspicuously white, covering the entire surface of the substratum. At 18° C. (Fig. 1, D) the

TABLE 1.—*Size of sclerotia of Sclerotinia minor and S. intermedia at different temperatures. In each case the maximum, minimum and average size of 50 sclerotia is indicated. Cultures at the higher temperatures matured much more rapidly than those at the lower. These were held in the refrigerator at 3° C. until the latter were mature. Sclerotia were measured at the end of five weeks*

Strain no.	Range in size and average size (in mm.) of sclerotia produced at different temperatures							
	25° C.		21° C.		18° C.		9° C.	
Sclerotinia minor Jagger.								
S2	1.0–5.0	2.34	0.75–4.0	2.06	1.0–3.0	2.0	0.75–2.5	1.4
S3	0.5–2.0	1.6	0.75–1.75	1.22	0.25–1.5	0.87	0.25–1.25	0.66
S86	1.0–4.0	2.04	1.0–3.5	2.0	0.75–2	1.41	———	——
S219	0.25–2.0	0.9	0.75–1.5	1.32	0.25–1.25	0.72	0.25–1.0	0.57
S334	1.0–5.0	2.95	———	——	0.75–2.0	1.2	0.25–1.75	0.7
S451	1.0–3.5	1.64	0.75–2.0	1.14	0.25–1.75	0.88	0.25–1.0	0.56
S452	1.0–2.5	1.51	1.0–1.75	1.34	0.75–2.0	1.24	0.25–1.25	0.6
Sclerotinia intermedia Ramsey.								
S127	0.75–2.0	1.3	1.0–3.0	1.84	2.0–4.25	2.8	2.0–6.0	3.62

sclerotia are not agglomerated, small, black, oval or spherical, with tendency to fuse, 0.75–3.0 mm., av. 1.54 mm. in diameter. The aerial mycelium is scanty, white, with substratum bare between flocci throughout the culture.

Race III includes S451 and S452. At 25° C. (Fig. 2, E) the sclerotia are relatively large, black, oval or spherical, with tendency to fuse, 1.0–3.5 mm., av. 1.58 mm. in diameter. The aerial mycelium is scanty, white and powdery near the center, disappearing toward the margin, at other times so scanty as to give the substratum a bare appearance throughout the area of growth. At 18° C. (Fig. 2, F) the sclerotia are small, black, oval or spherical, with tendency to fuse, 0.25–2.0 mm., av. 1.06 mm. in diameter. When the sclerotia are mature the aerial mycelium is evident only as minute flocci scattered over the substratum.

Attention should be called to the fairly uniform correlation in size of sclerotia, at different temperatures, of the strains of each of these races (table 1).

Relation of Temperature to Size of Sclerotia

Ramsey (3, p. 604), in describing the effect of temperature on sclerotia of species of *Sclerotinia* with which he worked, wrote as follows: "In general, when the temperature is near the minimum growing point for the fungus, the sclerotia have a tendency to be larger than the normal for room temperature. This is particularly so in the small sclerotial forms."

The strains which have been included within the three races already described have been grown at six different temperatures, ranging from 25° C. on the one hand to 6° C. on the other. *Sclerotinia intermedia* Ramsey was at the same time grown at these temperatures.

In cultures of all strains of *S. minor* studied it has been found that the sclerotia decrease in size with lower temperatures (Fig. 2, G and H and Fig. 3, I–M). This holds true from and including 25° C. to 9° C. (Table 1). At 6° C., although a copious growth of aerial mycelium usually occurs, few or no sclerotia are formed at the end of five weeks. On the other hand, in the case of *Sclerotinia intermedia,* the sclerotia are small (0.75–2.0 mm., av. 1.3 mm.) at 25° C., but steadily increase in size with each lower temperature (Fig. 4, N–Q)—just as Ramsey reports—until at 6° C. the sclerotia are larger than at any other temperature of the series (2.75–6.0 mm., av. 4.2 mm., table 1).

Ramsey's statement above quoted would appear, therefore, to apply to *S. libertiana* and *S. intermedia* only. If by the "small sclerotial forms" which he mentions in the second sentence he infers strains of *S. minor,* he would appear to be in error in holding that they tend to be larger at the lower temperatures. The writer fails to find in Ramsey's paper any experi-

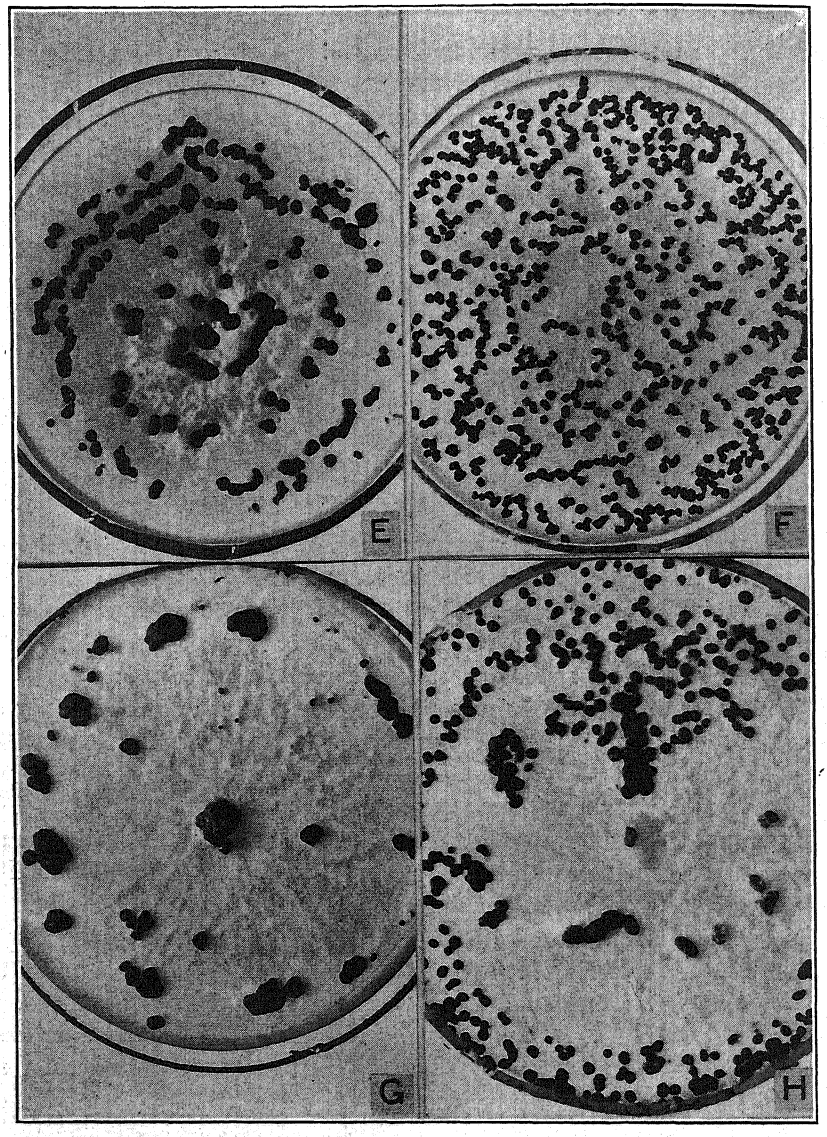


FIG. 2.—E and F. Cultures of *S. minor* (S452) showing the characteristics of race 3 when grown at 25° and 18° C. respectively.

G and H. *S. minor* (S219) grown at 25° and 21° C. respectively, representing the beginning of a series of cultures which illustrates the relation of the size of sclerotia to temperatures in this species.

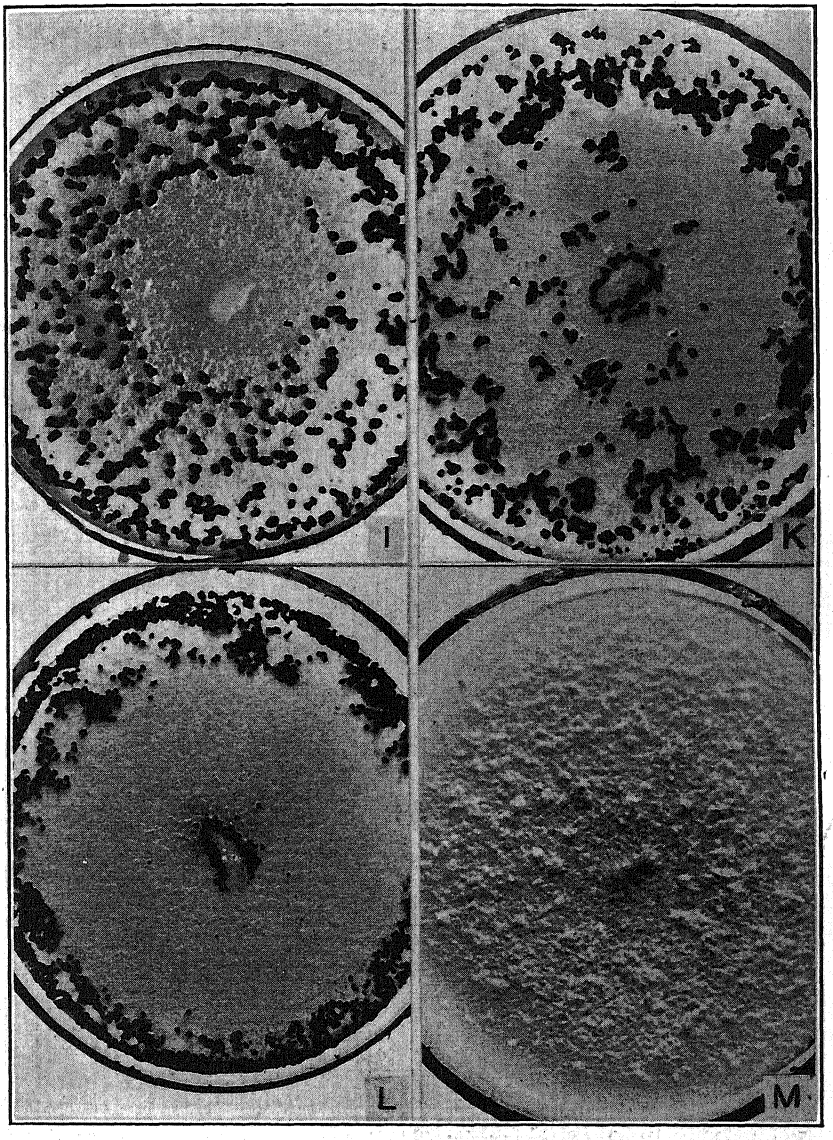


FIG. 3.—I, K, L, M. *S. minor* (S219) grown at 18°, 12°, 9° and 6° C. respectively, completing the series beginning with figures G and H, Plate II.

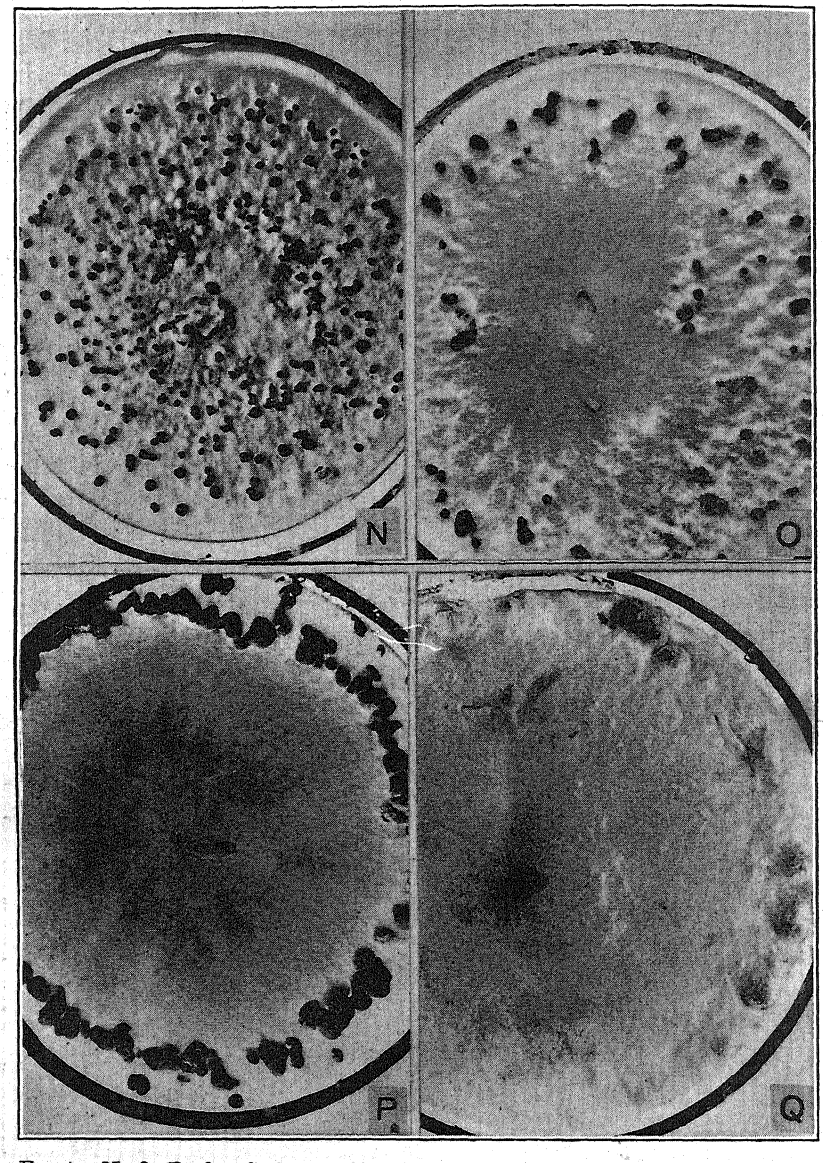


FIG. 4.—N, O, P, Q. *S. intermedia* (S127) grown at 25°, 18°, 12°, 6° C. respectively, representing a series of cultures which illustrates the relation of the size of sclerotia to temperatures in this species.

mental data which warrant such a conclusion. If by "small sclerotial forms" Ramsey refers to *S. intermedia* only, then his data and conclusions agree with what the writer has found for that species.

SUMMARY

It would appear from the results obtained that;

1. Characteristic differences occur among strains of the fungus now designated *Sclerotinia minor* Jagger, which for the present at least may be designated as racial characteristics.

2. The sclerotia of races of *S. minor* vary consistently in size with the temperature at which they develop; the lower the temperature the smaller the sclerotia.

3. The sclerotia of *Sclerotinia intermedia* Ramsey vary in size with the temperature at which they develop, but, in contrast with *S. minor*, the lower the temperature the larger the sclerotia.

These investigations were conducted in the laboratories of the Department of Plant Pathology at Cornell University during the fall term of 1927, while the writer was on sabbatical leave from Darmouth College. The writer is under many obligations to Dr. L. M. Massey, head of the department, for the use of the facilities of the laboratory and many other courtesies. The writer wishes to express his appreciation of Professor Whetzel's generosity in the use of his cultures and for his helpful suggestions during the progress of the work. The writer is also indebted to Mr. W. R. Fisher, photographer of the department, for the preparation of photographs.

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A MOSAIC DISEASE OF THE EASTER LILY

LAWRENCE OGILVIE AND CARL E. F. GUTERMAN[1]

INTRODUCTION

A transmissible virus disease of the Bermuda Easter lily, *Lilium harrisii* (*L. longiflorum* var. *eximium* Baker), and of certain Japanese varieties of *L. longiflorum* has recently been described by the senior writer (2)[2]. In addition to this disease, known as "yellow flat" and characterized by very marked downward curling of the leaves and dwarfing of the plants, another disease or group of diseases has been under observation in Bermuda and in greenhouses in the United States. Although our studies on this disease are as yet incomplete, it is thought advisable to present this preliminary paper.

The first exports of lily bulbs from Bermuda apparently took place in 1883 but there is good evidence that the Easter lily was grown in Bermuda as early as 1856. It is a native of the Liukiu Island, south of Japan, and probably reached Bermuda via the United States. About the year 1892 serious diseases made their appearance in the Bermuda lily fields but it was not until the end of the century that a marked decline of the industry became evident. From that time to within the last six years the industry has been, as remarked by E. H. Wilson (3), "a ghost of its former greatness." Details regarding the decline and subsequent resuscitation of the industry will be found in the senior author's paper on the yellow flat disease.

In 1897 Albert F. Woods, then assistant chief of the Division of Vegetable Physiology and Pathology, United States Department of Agriculture, presented a preliminary report of investigations (4), in which he described mainly the disease which is the subject of the present paper. He attributed the disease to weakening of the plants by improper selection and improper propagation, further increased by the attacks of mites, certain fungi and bacteria.

In 1901 Woods published further observations on the disease (5). An excellent colored illustration of an Easter lily affected with mosaic disease accompanies the article.

[1] The junior author is carrying out further investigations on this disease in pursuit of a joint project of The Horticultural Society of New York, The Department of Plant Pathology at Cornell University, The Boyce Thompson Institute for Plant Research, The New York Botanical Garden, and the Bermuda lily growers.

[2] Ogilvie L. A transmissible virus disease of the Easter Lily. Ann. Appl. Biol. **15**: 540–562. pl. 30–32. 1928.

Some further observations on the disease were made in 1915 when bulbs were forwarded to the United States Department of Agriculture by Mr. E. J. Wortley, then Director of Agriculture in Bermuda. Photographs of, and notes on, the appearance of the plants were prepared by C. W. Carpenter. These are now in the files of the Bermuda Department of Agriculture.

SYMPTOMS

The symptomatological picture presented by mosaic-affected lilies may take one of three following forms. These may or may not represent distinct diseases.

Form A. The form shown in Plate I, A, resembles closely the yellow flat disease. The lower leaves are in most cases normal. Above the third or fourth tier the leaves are curled downwards. In some cases they make a complete circle, the tips pointing upwards. The leaves have a shortened appearance due to the curling but are not actually shorter than normal. The color of the leaves is slightly chlorotic in longitudinal streaks usually about 5 mm. in length. As the leaves grow older these chlorotic markings give place to rusty necrotic areas, which ultimately coalesce. Withering of the diseased leaves takes place, as in the case of yellow flat, about two months before the normal dying of the plants. The apparently healthy leaves at the base remain green, while the others die from the base upwards. The characteristic streaking sometimes occurs on one side of the plant only.

The number of flowers is reduced and the flowers themselves are markedly distorted and sometimes show the chlorotic stripes and mottling characteristic of diseased leaves. The perianth segments are puckered and tend to adhere at the tips (Plate I, B). The stamens either do not develop anthers or are twisted and deformed. The pistil is much twisted.

Diseased secondary shoots are apparently always produced by such plants.

Form B. In the mild form pictured in Plate I, C, and illustrated by Woods (5), minute, pale green or yellowish, linear spots occur on the leaves. These dry out, giving rise to necrotic areas. There is not the pronounced chlorotic appearance or marked curling of the severe form *A*, but twisting of the leaves in various directions is common. In this form the flowers are not markedly distorted.

Form C. This form, illustrated in Plate I, D, is less common than the other two. It is characteristic, apparently, of the disease on certain strains of *L. harrisii*. The leaves have long tapering points and show, especially in the case of the young leaves, distinct mosaic-like mottling or chlorosis. In severe cases the leaves are markedly stiff, crack when twisted, and often

split or coalesce in pairs. The flowers are nearly always aborted, the flower parts remaining green and splitting (Plate I, E).

A somewhat similar splitting of the flowers, unaccompanied by the characteristic leaf symptoms, is produced by too rapid forcing or sudden changes of temperature in the greenhouse.

OCCURRENCE UNDER GREENHOUSE CONDITIONS

All three forms of mosaic have been observed by the writers on plants growing under greenhouse conditions. They are figured in the photographs mentioned above, which were taken in 1915 by C. W. Carpenter.

SEASONAL APPEARANCE

The first few leaves to appear above ground are apparently normal, as in the case of the yellow flat disease. Subsequently, the symptoms appear on the young leaves. The disease seems to be sensitive to slight changes in temperature. A mean shade temperature of 70° F. or higher tends to mask the symptoms to a certain extent. Twisting is not pronounced and the chlorotic areas become much less marked. If cold weather supervenes, the twisting and chlorosis then occur on all new growth.

From flowering time onward, the symptoms in the field are greatly masked by the increase in thickness and color of the leaves. In severely affected plants, dying of the leaves from the base upwards takes place shortly after flowering time, as in the case of yellow flat.

The disease is most noticeable in shaded situations, *e.g.*, at the edges of fields or among grass. The above statements apply to all three forms of mosaic here under consideration.

SPREAD OF THE DISEASE

Evidence of the spread of the disease is clearly seen in the Bermuda lily fields from the month of January onward. The symptoms appear on the topmost leaves of plants whose lower leaves are apparently quite healthy. Most of the spread takes place during the cooler months of the year when the prevailing temperature is about 60°–65° F. Cases have been observed in which the disease spreads in the greenhouse as well.

The following insects occur most commonly in the lily fields of Bermuda: *Aphis gossypii* Glover, *Aphis ogilviei* Theobald, *Empoa fabae* Harr. (=*Empoasca mali* le Baron), and *Cicadula sexnotata* Fall. No positive results have yet been obtained in the attempted transfer of the disease to healthy plants by means of these insects, by means of the bulb mite (*Rhizoglyphus hyacinthi* Banks), by inoculation with extracted juice, or by rubbing healthy plants with mosaic leaves.

Spread of the disease in the fields is somewhat irregular and does not occur in circular areas, as in the case of yellow flat.

EFFECT ON THE BULBS

The disease causes a slight reduction in size and a flattening of the bulb similar to that caused by yellow flat. The mild form (*B*) has little effect on the bulb. For this reason bulbs affected with this form of the disease are not detected in the packing houses and are therefore shipped.

CONTROL IN THE FIELDS

Successful control of the disease has been achieved in the Bermuda lily fields by careful roguing as soon as symptoms make their appearance on the plants. Many of the stocks there are now remarkably free from mosaic infection.

It is a common custom of the better growers to mark their best and most healthy plants for "shelling down," the shells from these yielding healthy stock.

An inspection of the lily fields is now carried on by the Bermuda Department of Agriculture from March onward. This year (1928) not more than 5 per cent of mosaic plants were allowed in the fields. Growers whose stock showed higher than 5 per cent of mosaic plants were not allowed to export their bulbs.

DISCUSSION

It is probable that the disease here reported should be classified as a virus disease of the mosaic type. This is suggested by (a) the presence of mosaic-like chlorotic areas, (b) the susceptibility of diseased plants to changes in temperature, high temperatures tending to mask the symptoms somewhat, (c) its occurrence in certain strains of lilies, (d) the apparent non-recovery of affected plants, (e) the efficiency of roguing in its control, (f) its similarity in several respects to yellow flat, a transmissible virus disease, and (g) its similarity to a mosaic disease of *Lilium auratum* Lind., which has been shown to be transmissible (1).

SUMMARY

A virus disease of the mosaic type is reported on the Bermuda Easter lily (*Lilium harrisii*).

The symptom pictures of three distinct forms *A*, *B*, and *C* are described. These may or may not represent distinct diseases.

The disease has been observed under greenhouse conditions as well as in the field. The symptoms are partially masked by temperature higher than 70° F.

Observations in the field and in greenhouses give clear evidence that the disease spreads from mosaic to healthy plants. The spread occurs irregularly rather than in circular areas around points of early infection.

Attempts to transmit the disease by means of the following insects have failed: *Aphis gossypii, Aphis ogilviei, Empoa fabae,* and *Cicadula sexnotata.* Negative results were obtained with the bulb mite *Rhizoglyphus hyacinthi.* Attempts at mechanical transmission have also failed thus far.

Successful control of this disease has been achieved in Bermuda by means of careful roguing and by the use of healthy plants for propagation.

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AND
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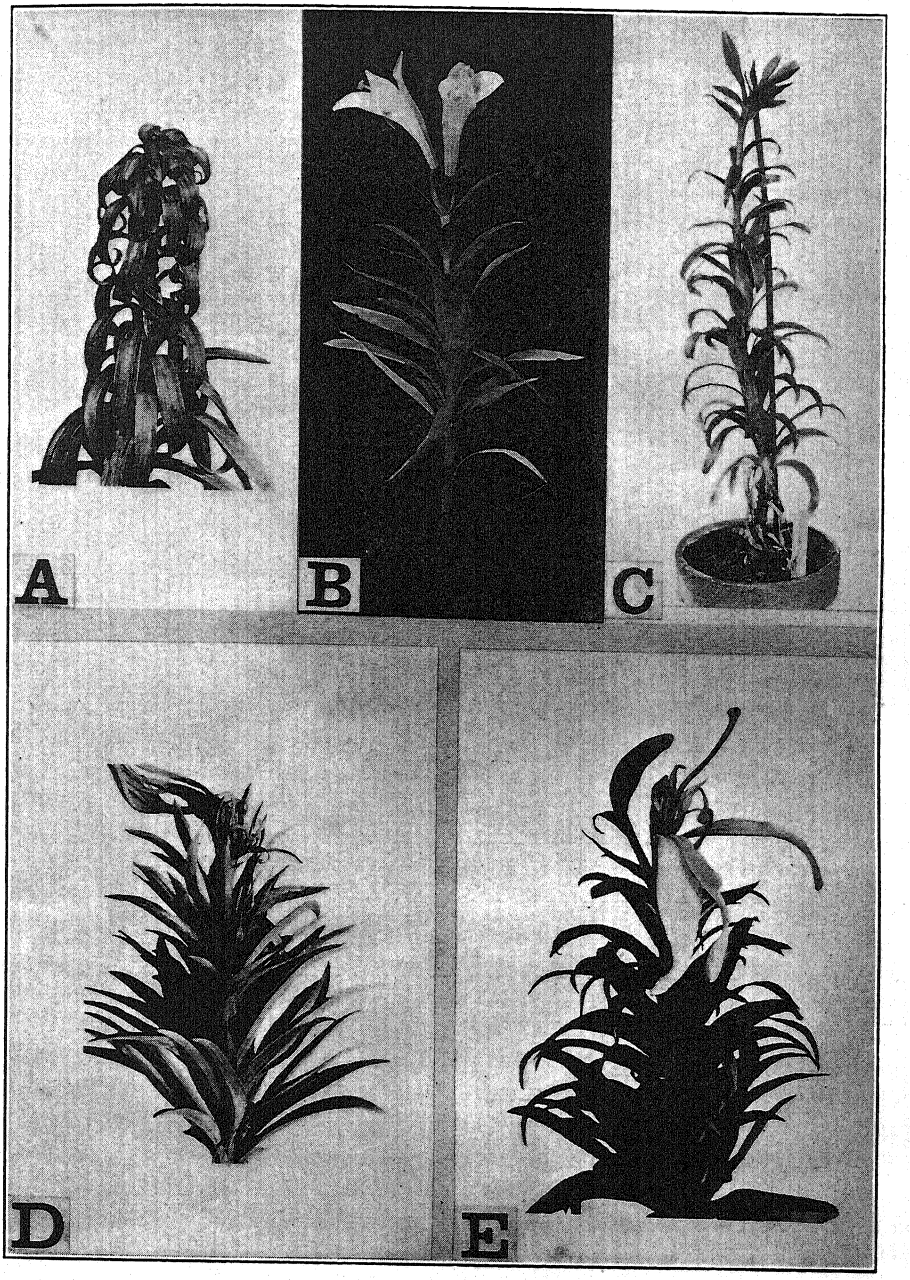
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EXPLANATION OF PLATE V

Mosaic plants of *Lilium harrisii.*

A: Plant affected with mosaic *A* or the severe form. B: Typical flower distortion as a result of infection with mosaic *A*. Plant grown in a greenhouse at 75° F. Note absence of marked twisting of leaves. C: A greenhouse-grown plant affected with mosaic *B* or the mild type. D: Plant affected with mosaic *C*. E: Flower distortion and splitting typical of mosaic *C*.


A
B
C
D
E

PHYTOPATHOLOGICAL NOTES

An undescribed leaf condition associated with damping-off diseases of sugar-beet seedlings.—Healthy sugar-beet seedlings have erect straight petioles, while plants injured by damping-off diseases commonly develop bowed petioles (Fig. 1). The bowing of petioles, although beginning with

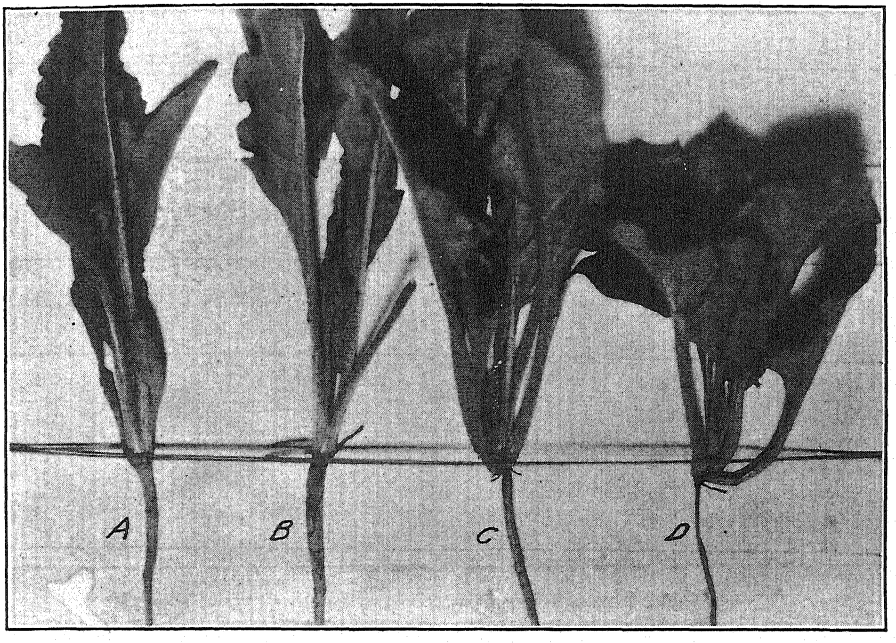


FIG. 1. A and B, healthy sugar-beet seedlings with straight petioles. C and D, diseased sugar-beet seedlings with bowed petioles. The bowing is the result of root infection commonly referred to as black root or damping-off.

the early injury of plants, is most pronounced in the advanced stages of these diseases. The bowing is greatest at the base of the petiole and results in a more open type of top than is common to healthy sugar-beet seedlings.

Damping-off diseases have been known to be the most serious source of injury to sugar-beet seedlings since the beginning of the industry. Such infection is commonly called black root, descriptive of the blackened and discolored roots of diseased seedlings (Fig. 2). The literature dealing with black root of sugar-beet seedlings, based upon observations and investigations in the United States and foreign countries, is voluminous, and no attempt here is made to review it beyond stating that blackened roots, retarded growth, and a pale leaf color have always been noted as the principal symptoms. No reference has been made heretofore to the association of bowed petioles with damping-off diseases.

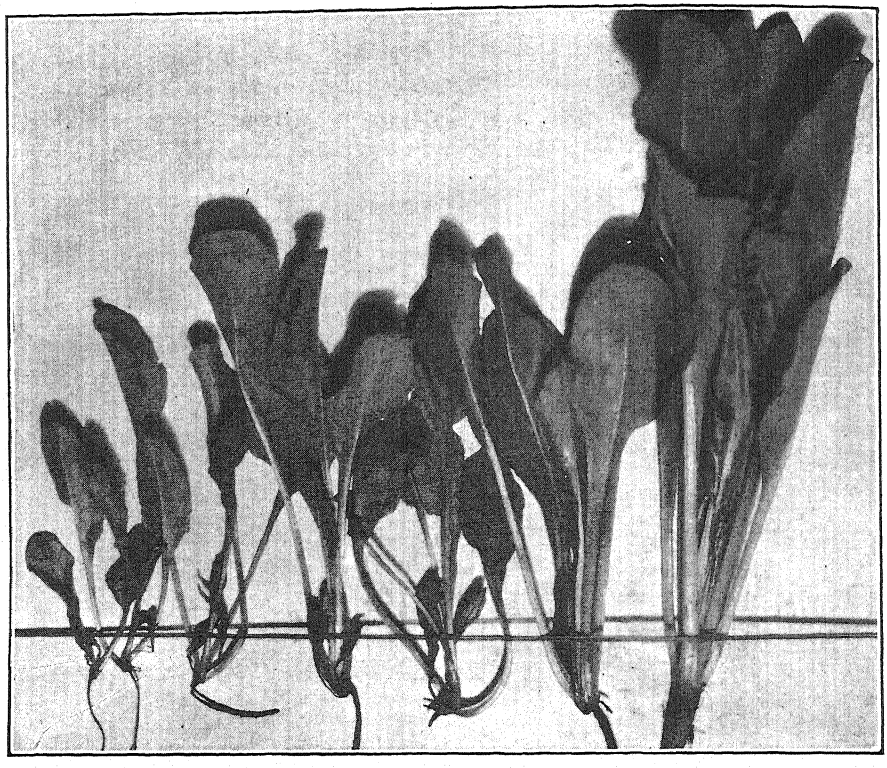


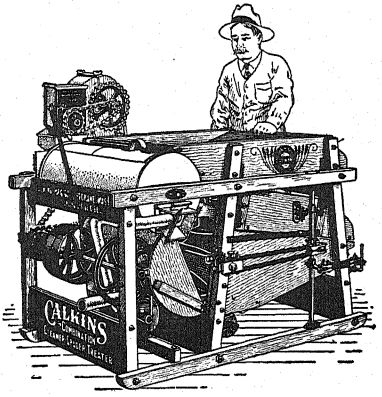
FIG. 2.—Sugar-beet seedlings of the same age. The large seedling at the extreme right is healthy. Note the straight petioles and vigorous growth. The other six seedlings show various degrees of injury by black root or damping-off. Note the conspicuously bowed petioles, blackened roots, and small size of plants.

Sugar-beet seedlings with booked petioles are easily recognized. When the seedlings have from 4 to 6 leaves it is necessary to examine the petioles closely, but when there are from 8 to 10 leaves plants injured by damping-off diseases can be recognized at a distance of several feet.

The writers have selected several thousand beets with bowed petioles from fields in various parts of the western United States and have found black-root injury on each of them.

This method of identification of sugar-beet seedlings injured by damping-off diseases is of practical importance, inasmuch as injured plants can readily be identified without pulling the seedlings from the ground. Sugar-beet thinners can easily remove diseased plants and leave the healthy ones. Very satisfactory results have been obtained by instructing commercial thinners in this means of identification: they soon left very few bowed seedlings. The value of removing diseased seedlings and leaving the healthy ones is very apparent.—S. B. NUCKOLS and C. M. TOMPKINS, Office of Sugar Plants, Bureau of Plant Industry, United States Depart-

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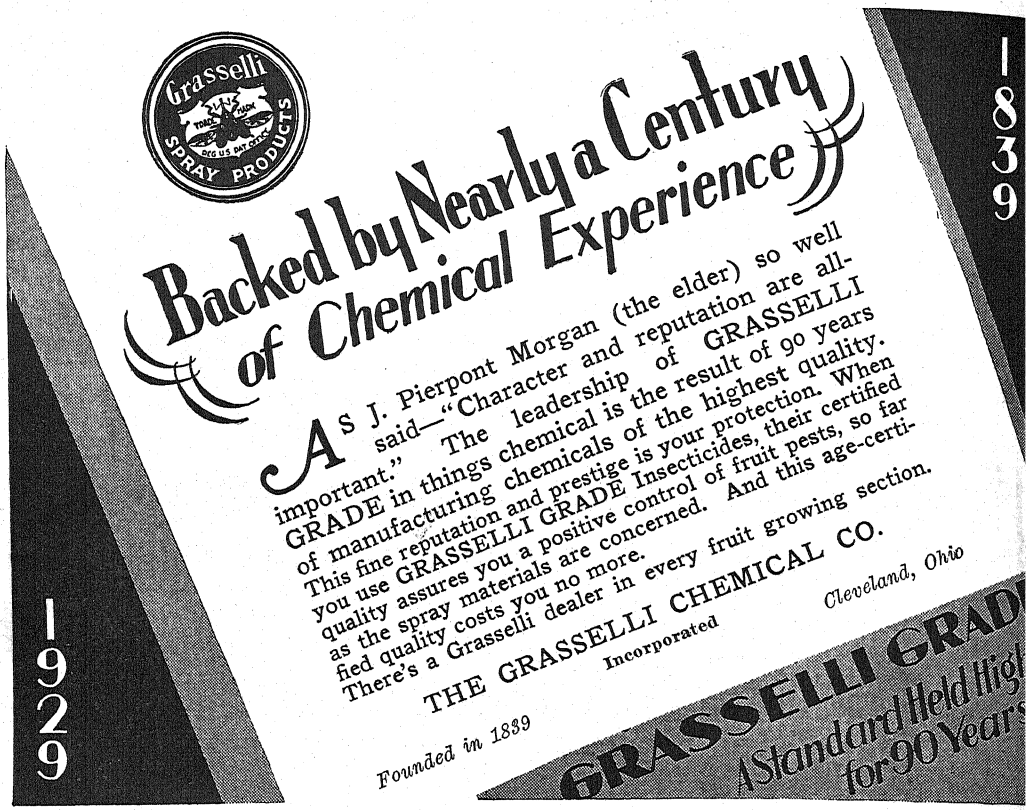
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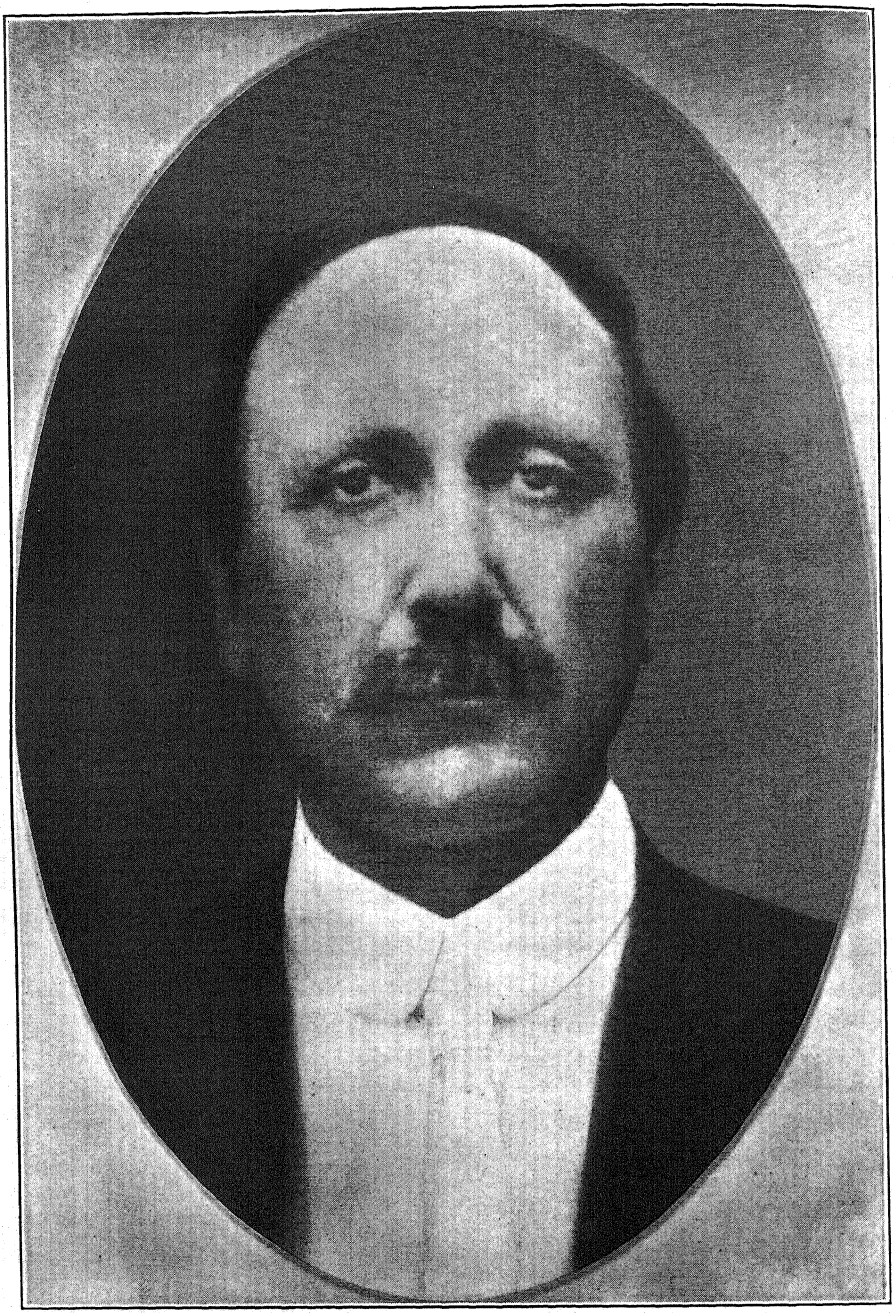
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MARK ALFRED CARLETON

PHYTOPATHOLOGY

VOLUME 19 NUMBER 4

APRIL, 1929

MARK ALFRED CARLETON
1866–1925

E. M. FREEMAN

The death of Mark Alfred Carleton, in Paita, Peru, on April 26, 1925, marked the passage of a great pioneer in more than one field of botanical and agricultural science. Carleton's years were singularly eventful. He made several excursions into foreign lands, his scientific contributions covered a wide range of subjects, and his scientific activities developed important contacts with industrial and agricultural enterprises. He was primarily a man of action and packed into the fifty-nine years of his life an amazing record of achievements. Born (1866) near Jerusalem, Ohio, he passed his boyhood in the stern environment of the pioneer agriculture of that day, which left on his character its impress of ruggedness and a lifelong appreciation and sympathy for the farmer of the great middle west. This attitude was further accentuated by his college and scientific training in midwestern institutions of higher education.

The detailed story of Carleton's life has been presented in several biographical sketches (see bibliography) and need not be repeated here. The unfortunate events of the last years of his administrative career as Cerealist of the United States Department of Agriculture and the distressing struggles of the succeeding years, which ended in his death in far-off Peru, have aroused partisan sympathies which make, perhaps, a fair and just appreciation of Carleton's place in history difficult at this time.

Carleton's serious scientific career began in Kansas with his work on the diseases of cereals, and especially on the rusts of small grains. From 1893 to 1905 he published numerous contributions on the grain rusts of the United States. The classic work of Jakob Eriksson in Sweden had demonstrated the complexity of physiologic-form relations in European grain rusts. Carleton laid a firm basis for a similar demonstration of the physiologic-form relation in American rusts of cereals. It is chiefly upon these contributions that Carleton's achievement as a plant pathologist must rest. Although this work was of a pioneering nature, it was comprehensive and exhaustive, has stood the test of time, and will stand as one of the significant contributions to American phytopathology.

To a man like Carleton, scientific investigations required early translation into agricultural practice. The need for wheats resistant to rust and adapted to the Great-Plains region became to him a dominant problem. The rapid expansion of the personnel of the Office of Cereal Investigations from about 1905 to 1910 gave him the opportunity of attempting to meet this and many other problems of small-grain improvement. From this point on, his interests and activities became chiefly those of the agronomist. Probably in this field lie Carleton's greatest contributions to science and agriculture. His explorations and field observations and the extensive introductions and field-plot experiments of the last ten years of his administration of the Office of Cerealist made some of the really great contributions to American agronomy. The introduction of rust-resistant and drought-resistant durums and of winter-hardy Turkey wheats are only two of the many outstanding achievements of this period of his work. The office of which he was chief introduced hundreds of varieties of small grains, tested them in all of the potential grain-growing areas of the United States, inaugurated field studies covering wide ranges of ecologic relations, studied small grains from almost every scientific and practical angle, and undertook basic and applied studies on the breeding of cereal crops.

Carleton's work as general manager for the Pennsylvania Chestnut Blight Commission in 1913–14, one of the few excursions away from the field of agronomy, and those contributions subsequent to his resignation as Cerealist of the United States Department of Agriculture in 1918, chiefly in the field of tropical plant pathology, constitute his minor scientific achievements. The latter were made under stress of financial difficulties, in an unfriendly tropical environment, and in a field foreign to his earlier experience.

Of Carleton as a scientist, the outstanding characteristics were a remarkably keen faculty for field observations and an equally remarkable ability to retain such facts. He was independent and bold in his thinking, and slow to recede from a position once taken. He had a wide knowledge of the literature of his field and was particularly rich in his knowledge of field conditions. He had a large research curiosity but was not satisfied with scientific results until applied in practice. The last ten years of his administration of the Office of Cerealist was a tremendous growth and expansion in the office and field personnel and activities. Carleton was not, by nature, an administrator. He was rather the field investigator. Unfortunate financial ventures and serious illness in his family, in addition to the demands of a rapidly growing and complex organization—demands that were irksome in contrast to his natural inclination for personal field study—gradually brought on difficult, if not impossible, situations. His resigna-

tion from the Office of Cerealist in 1918 was the culmination of one of the not infrequent tragedies in the history of science. Ambition and enthusiasm of man may build a machine that outgrows his power to direct and control.

The administrative rock on which Carleton's career was wrecked is of historical interest only as the agency which terminated the most important phase of his scientific contributions. It can in no wise detract from the real and significant contributions which he made to agronomy, to plant pathology, and to practical agriculture, as well as those of minor importance which he made in other branches of plant science. He was one of the greatest pioneers in American agronomy and deserves a high rank among the pioneer plant pathologists of this country.

As a man, Carleton had a personality that held many staunch friends and developed numerous enemies. To those who knew him intimately, he exhibited a lovable and friendly personality. He was simple and unostentatious in manner but was sometimes lacking in tact. He was firm in his convictions but was not blind either to argument or contrary evidence. He was an untiring and persistent worker. His large size and slow physical actions concealed an unsuspected alertness of mind. He was a bold dreamer in scientific theories. In practical affairs his optimism frequently got the better of his judgment. His dominant characteristic was an untiring and persistent will to work and an uncompromising and unyielding persistence in the pursuit of his cherished projects.

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EXPERIMENTS WITH REPEATING PINE RUSTS

E. P. MEINECKE

FOUR FIGURES

INTRODUCTION

In two former papers (8 and 9) the writer has discussed his experiments with repeating pine rusts. In 1916 he reported the first successful transfer of the aecial stage from pine to pine, under controlled conditions, with omission of an alternate host and of the uredinial and telial stages. At the same time Haack (4) had been conducting a series of independent experiments with *Peridermium pini* in Germany, the results of which reached this country late on account of the war. Unfortunately Haack had performed all his inoculations in the open, in the infected forest, and had selected, for his experiments, trees already heavily infected with *P. pini*. His deductions, namely that *P. pini* can be transferred directly from pine to pine through aeciospores, were therefore not valid. No alternate host for *P. pini* is known. Haack's work found a first reaction in a review by Fischer (3) who reported it with critical reservations. Haack's paper was discussed by Tubeuf (11). He emphasizes the inconclusive character of Haack's results and reports (11, p. 274) unsuccessful inoculations of his own with the same rust on *Pinus silvestris* and *P. montana* (1915 and 1916). The writer's positive results, obtained with a rigidly controlled technique, would in themselves have lent strong probability to Haack's findings, had not Klebahn (7), unaware of the fact that the problem had been definitely solved, taken up Haack's work and repeated it under controlled conditions. He proved that the aeciospores of *Peridermium pini* are capable of infecting *Pinus silvestris* directly. *Peridermium pini* is, then, the second example of this exception to the general rule of heteroecism in the genus Cronartium, the Peridermium on *Pinus radiata* investigated by the writer being the first. A short paper by Tubeuf (12) refers again to Haack, who in the meantime had fallen in the war, and accepts Klebahn's conclusions.

Owing to the war blockade neither Haack, Klebahn nor Tubeuf knew of the work of the writer. Tubeuf's papers finally arrived in this country some time after the war was over. The writer's second publication on the subject (8) gave the results of a series of experiments in corroboration of his first paper, and reported similar success with a third Peridermium (*Cronartium harknessii*, nov. comb.).

The writer's work dealt with a Peridermium on *Pinus radiata* and a few other pines of the coastal region and with a Peridermium confined to the

pines of the interior mountain regions of California, such as *Pinus contorta, P. ponderosa* and *P. sabiniana.* The Peridermium on *Pinus radiata* was first considered to be *Peridermium harknessii* Moore. On the strength of inoculation experiments on oaks reported by Hedgcock (10) the rust was later classed as *Peridermium cerebrum* Peck (*Cronartium cerebrum* (Peck) Hedg. and Long), particularly since uredinial and telial forms are common on oaks in the *Pinus radiata-Quercus agrifolia* association near the seashore in California. Hedgcock's results had been meager and in a recent personal communication to the writer Hedgcock himself doubts the validity of the experiment. Without wishing to discuss at this time the question of the true identity of the rust, the writer proposes to refer to this rust in the following no longer as *Peridermium cerebrum* Peck but *Peridermium cerebroides* (*?*). It seems probable that we have to do with a new form for which a valid name is lacking. It is not impossible that the rust may have lost altogether its faculty to infect any of the plants now available within the range of its aecial hosts. The same seems to be true of *Peridermium pini.* The other Californian pine gall rust, apparently confined to mountain pines, is designated by the writer as *Peridermium harknessii* (*Cronartium harknessii,* nov. comb). Arthur (1) seems inclined to place this rust under *Cronartium coleosporioides* (Diet. and Holway) Arth. and to maintain the presence of *Cronartium cerebrum* (Peck) Hedgcock and Long on the Pacific Coast. The writer now believes that the common gall-forming Peridermium on *Pinus radiata* is not *Peridermium cerebrum.* He still believes that *Peridermium stalactiforme* is a synonym of *P. filamentosum. Cronartium coleosporioides,* in the writer's opinion, comprises one or two, perhaps even three non-gall-forming Peridermia. In order to avoid further confusion he will refer in the following to the rust which causes galls on *Pinus contorta, P. ponderosa* and other pines of the mountain regions of California as *Cronartium harknessii.*

INOCULATION EXPERIMENTS

All the experiments reported in the following were carried out at the Feather River Experiment Station of the United States Forest Service near Quincy, Plumas Co., Calif. Quincy lies at an elevation of 3600 feet in the northern extension of the Sierra Nevada. The Station is located in a forest composed of *Pinus ponderosa, Pinus lambertiana, Pseudotsuga taxifolia, Abies concolor* and *Libocedrus decurrens,* an association in which *Pinus ponderosa* represents the character tree. The location is several hundred miles distant from the nearest host of *Peridermium cerebroides* (*?*). *Peridermium harknessii* is exceedingly rare. In the course of ten years only a single gall has been observed, in spite of consistent and careful search. This

gall, found in 1918 on a young Western yellow pine, was immediately destroyed. *Castilleia miniata,* its most common alternate host, is at home in the vicinity, but the rust has never been found on it. A greenhouse and a nursery offer excellent conditions for clean and controlled work. The methods of inoculation used were either that of introducing aeciospores into tangential slits of the type described previously (8, p. 286) or that of showering the plants with aeciospores. In the latter case the plants were first sprayed with water and the spores were either dusted over the plants or sprayed on, suspended in water. After inoculation the plants were maintained for several days in a moist atmosphere of even temperature by the use of bell jars or portable inoculation chambers made of panes of celluloid and cheesecloth nailed on light wooden frames.

PERIDERMIUM HARKNESSII

The identity of the material used was proved by the successful inoculation of plants of *Castilleia* (*miniata?*) with aeciospores from typical galls on *Pinus contorta* and *P. sabiniana.*

Slit Method. Table 1 gives the results of inoculations made in the greenhouse by the introduction of aeciospores into tangential slits through the epidermis and cortex layers of the main stem of young pines. Of a total of 113 inoculated slits 79, or 70 per cent, developed definite galls. The

TABLE 1.—*Infections by the slit method. Peridermium harknessii*

	Slits	Positive	Negative
1. *P. contorta–P. contorta*	16	11	5
2. *P. contorta–P. radiata*	32	32	.....
3. *P. sabiniana–P. radiata*	12	9	3
4. *P. sabiniana–P. jeffreyi*	12	9	3
5. *P. sabiniana–P. coulteri*	8	5	3
6. *P. (contorta) contorta–P. contorta*	10	7	3
7. *P. (jeffreyi) contorta–P. jeffreyi*	15	4	11
8. *P. (jeffreyi) contorta–P. radiata*	8	2	6
	113	79	34

last four inoculations are of particular interest. For No. 5 (*Pinus sabiniana-P. coulteri*) small seedlings of about 2″ or 5 cm. in height were used. The seeds had been sown in the greenhouse in November, 1919, and the inoculation took place in April, 1920. The extreme tenderness of the thin young stems makes the incision as well as the insertion of spore material a delicate performance. The positive results obtained in over 62 per cent of the slits show that even very young tissues are susceptible to infec-

tion. Each plant could receive not more than one slit. Two years after the inoculation one of the plants had attained a height of 16 cm. and bore a gall measuring 7.5×1.2 cm., accompanied by a well-developed witches'-broom. The experiment adds *Pinus coulteri* to the list of host plants artificially infected with *Peridermium harknessii*. Nos. 6, 7 and 8 were made with pedigreed material, the result of former inoculations by direct transfer of aeciospores, so that the resulting galls represent the third generation under control. While No. 6 (*Pinus* (*contorta*) *contorta-contorta*) shows 70 per cent success, the No. 7 and 8 (*P.* (*jeffreyi*) *contorta-jeffreyi* and *P.* (*jeffreyi*) *contorta-radiata*) were successful only in 26 and 25 per cent of the inoculations. Moreover No. 6 developed very slowly.

All check plants remained free from infection.

Spore Showers. In the first paper of this series the writer had expressed the opinion that the infection of pines with aeciospores took place largely through wounds, caused probably by insects. In order to verify or disprove this assumption he attempted to inoculate young pines by showering them with aeciospores. The plants were apparently free from insects and had been carefully handled in order to avoid accidental wounding. The method is not new in its application to repeating rusts on pines. Klebahn (7, p. 200, 201) had dusted young specimens of *Pinus silvestris* with aeciospores of *Peridermium pini* and had obtained galls on unwounded plants, and Tubeuf (11, p. 274) reports similar but unsuccessful experiments.

The writer's inoculations by the method of showering the plants with aeciospores were carried out partly in the greenhouse, and partly in the nursery. In the latter case the plants were protected and isolated for several weeks with bell-jars or portable inoculation chambers. In spite of the fact that the region of the Experiment Station is, apparently at least, practically free from *Peridermium harknessii* the results of the nursery experiments would not be valid, had not the fact of direct aecial transfer been abundantly shown by greenhouse experiments and by the fact that of the many hundreds of plants in the nursery no galls appeared except on inoculated plants. As in the greenhouse, all galls showing the beginning of sporulation were either at once removed or carefully wrapped in cheesecloth to prevent an accidental transmission of spores. The results obtained in the nursery have therefore the value of corroborative data. Of 14 pines showered in the greenhouse 11, or 78.5 per cent, became infected and developed 47 galls or an average of 4 galls per plant. Of 18 pines showered in the nursery 8 plants, or 44 per cent, developed 27 galls, averaging a little over 3 galls per plant.

Peridermium harknessii is known to go to *Castilleia sp.*, an alternate host. One could think of a differentiation of the rust species into strains,

one of which had retained the faculty of heteroecism while the other had lost it. That this explanation has no foundation was proved by inoculation of *Castilleia* (*miniata?*) with the same aeciospore material which was used for Nos. 1, 3, 4 and 5 of table 1. All of these inoculations, made in the usual manner in the greenhouse, were successful, reaching the uredinial, and in one plant, the telial stage. The check plants remained uninfected.

Peridermium cerebroides (*?*)

The inoculations with this species were made in the greenhouse, under the usual precautions. Their main objective was to ascertain whether this Peridermium required wounding of the host as a condition for entrance. Four plants of *Pinus radiata* were sprayed with water. The spore dust of *Peridermium cerebroides* (*?*) from *Pinus radiata* was then applied with a sterile brush. Of the four plants three became infected and produced 12 galls or an average of 4 galls per plant. Fig. 1 illustrates the location



FIG. 1. Typical galls of *Peridermium cerebroides* (*?*) developing on *Pinus radiata* as a result of showering the plants with aeciospores.

and type of galls produced. The large plant to the right has escaped infection. One of the infected plants died and was removed.

Peridermium occidentale, P. filamentosum,
P. pyriforme.

All attempts to transmit *Peridermium occidentale, P. filamentosum* or *P. pyriforme* from one pine to another by the use of aeciospores failed to

produce results. All plants were kept under observation for at least two years. The details are given in the following tabulation:

Peridermium occidentale.

Slit method: *Pinus edulis-P. monophylla* 23 slits inoculated.
" *monophylla-P. monophylla* 9 slits inoculated.
Spore shower: " *monophylla-P. monophylla* 6 plants inoculated.

On three of the latter plants necrotic spots appeared without developing into more significant stages. These experiments should be repeated.

Peridermium filamentosum.

Inoculations were made from *Pinus jeffreyi* to *P. jeffreyi, P. contorta* to *P. contorta* and *P. ponderosa* to *P. ponderosa.* Two plants were showered with spores and 59 slits were inoculated. All experiments were unsuccessful.

Peridermium pyriforme.

In the attempt to transmit the rust directly from *Pinus ponderosa* to *P. ponderosa* 28 slits and incisions were inoculated and three plants were showered with aeciospores. The experiments remained without results after being under observation for six years.

INCUBATION PERIOD

The length of the incubation period varies greatly. Undoubtedly the condition of the inoculum and of the plant inoculated as well as the climatic conditions under which the infected plant is kept are important factors. The time of inoculation, the proper synchronization of the physiological state of the inoculum with that of the new host must surely have their influence upon the establishment of the rust within the plant. But of all factors which determine the length of the incubation period the relative susceptibility of the species and of the individual plant is perhaps the most weighty. Of all these relationships very little is known. The only data available, meager at best, are of a statistical nature.

In our experiments, measurements were taken on practically all surviving galls in 1920 (September, November), 1921 (April, December), 1922 (May, November), 1923 (April, August, December), 1924 (July, November), 1925 (May, June, October), the last-named year being the one in which the Experiment Station was abandoned. The series of inoculations here reported began in 1919. From that year onward the plants were kept under observation. An exact determination of the incubation period is impossible except by microscopical examination which entails destruction of, or at least serious interference with, the plants. Macroscopic examina-

tion of the initial stages, indicated by very slight swellings, is likely to be misleading. Furthermore, the great distance of the Experiment Station from headquarters in San Francisco eliminated the possibility of systematic and continued observation, so that the data here presented on the length of the incubation period are approximations. Since the Station was frequently visited during the period from spring to fall the plants were more closely watched during this time than during winter. The high mortality in the inoculated plants introduces a further weakness in the statistical material.

Peridermium harknessii.

Slit method. All inoculations were made in the greenhouse. The incubation period is here understood as the length of time elapsing between the date of inoculation and the first appearance of swellings indicating infection. The incubation periods expressed in months, as given in table 2, show

TABLE 2.—*Incubation period. Peridermium harknessii. Slit method.*

Number of swellings	Incubation period
6	3 months
21	4.6 "
2	8.5 "
2	10.3 "
3	13.6 "
32	14 "

great variation, although all plants were kept through the years under identical conditions. The type of host plants used varied very little. They were young potted plants ranging from about one-half to about two feet in height, with the exception of the series of very small seedlings of *Pinus coulteri* which has been referred to before.

The hosts used in this series were as follows: *Pinus sabiniana* to *P. jeffreyi, P. sabiniana* to *P. radiata, P. sabiniana* to *P. coulteri, P. contorta* to *P. contorta, P. (jeffreyi) contorta* to *P. contorta* and *P. (contorta) contorta* to *P. contorta.* The two last named are pedigreed, the name in the parentheses indicating the origin of the material used.

Spore shower. In this series part of the inoculations were made in the greenhouse, and part in the nursery. Since the conditions in the latter undoubtedly come closest to those prevailing in the open forest, one would expect the results to differ from those obtained under the artificial conditions of the winter-heated greenhouse. Table 3 gives the incubation period for the inoculations in the greenhouse and in the nursery. It may be well

to repeat that none of the hundreds of check plants in the nursery ever became infected. The following host plants were used in the transfers: *P. ponderosa-P. ponderosa, P. contorta-P. ponderosa, P. ponderosa-P. jeffreyi.* The aeciospore material employed in the greenhouse experiments came in part from Idaho, in part from British Columbia. All the aecial material used in the nursery tests came from Colfax, California, where *Peridermium harknessii* was first discovered. The only significant difference between the greenhouse and nursery sets consists in the uniformly much longer incubation period prevailing in the nursery. Obviously the artificial conditions in the greenhouse favor an earlier and more rapid development of the mycelium. All the nursery inoculations were performed on the same date (May 24, 1921). For the greenhouse experiments, with one exception (1 gall, 13.6 months' incubation), Idaho material was used. Inoculations made with the same aecial material show remarkably uniform results.

TABLE 3.—*Incubation periods in greenhouse and nursery. Peridermium harknessii.*

	Plants used	Swellings	Incubation period
Greenhouse	8	38	5 months
	5	7	11 "
	1	1	13.6 "
Nursery	6	23	23.5 "
	1	1	27 "

Peridermium cerebroides (*?*).

Slit method. Only a few inoculations by the slit method were made at the Experiment Station, all of which failed. It had been attempted to transfer the rust from *Pinus radiata* to *P. virginiana, P. thunbergii* and *P. densiflora.* Whether the failure was due to the lack of susceptibility of the hosts tested or to the very poor condition of the stock available remains uncertain. No swellings and galls developed.

Spore shower. A good series of galls was obtained by showering four plants in one pot (see Fig. 1). One of the plants remained sound. On the remaining three plants 13 galls developed which were observed and periodically measured through three years. The transfers were made in the greenhouse from *Pinus radiata* to *P. radiata,* the commonest host for *Peridermium cerebroides* (*?*). The inoculations, made on three plants, dated from March 27, 1920. On the first plant two swellings appeared after six months. The second pine showed four swellings after six months and a fifth swelling developed after more than twenty months from the date of inoculation. The third pine had five swellings six months after inoculation,

to which a sixth was added two months later. This plant died and is therefore not shown in Fig. 1.

GROWTH RATE OF SWELLINGS AND GALLS

The tabulation of growth figures is often difficult. Two neighboring galls on the same stem may grow together into one, or the swellings may extend into the branches or from the branches into the stem. New galls may appear from latent infections. Frequently the galls are so irregular in shape that their measurement rests upon uncertain ground. The majority of the swellings, however, develop according to type, forming galls of regular form and of the well-known shape.

Peridermium harknessii.

It would serve no purpose to give here the growth history of a great number of individual galls. In Fig. 2 the averaged measurements for three

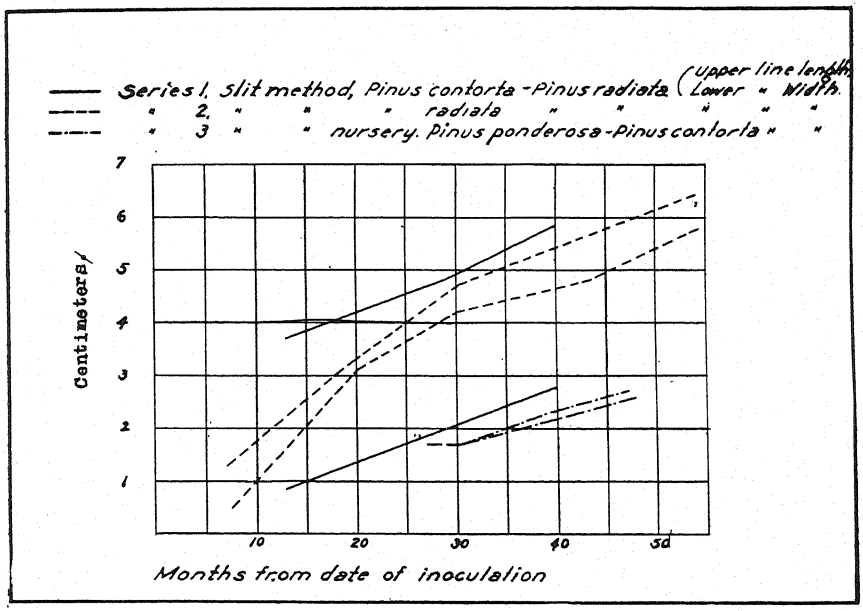


FIG. 2. Growth rate of galls, *Peridermium harknessii.*

series are given in centimeters over months elapsed since the date of inoculation. The upper lines represent height, the lower width. The figure shows plainly that the rate of growth for the galls grown in the nursery lags behind that of the greenhouse series. The highest of the averaged figures runs to 6.4×5.7 cm. after 54 months. Individual cases exceed these figures. One gall grew in 40 months to 9.0×2.7 cm., another in 61 months to 8.7×7.9

cm., and a third in 72 months to 7.4 × 6.8 cm. The most rapid growth was made by one gall which attained 18.4 × 1.4 cm. in 28 months.

Peridermium cerebroides (*?*).

No data on experiments by the slit method are available. As reported above only a few inoculations were made, all of which failed to produce galls.

Spore shower. In a series of experiments (*Pinus radiata-P. radiata*) conducted in the greenhouse (see Fig. 1) three out of four trees developed galls. The measurements of the galls were averaged and are presented below. The data are slightly disturbed through the late appearance of two new galls from latent infections. The inoculation took place on March 27, 1920; 7.6 months later the average dimensions were 1.4 × 6 cm. After 20.3 months they were 2.7 × 1.8 cm., and after 25.6 months 3.2 × 2 cm.

FORM OF THE GALLS

The first result of infection appears in the form of slight swellings which, as a rule, soon become more pronounced and finally grow into typical galls. As may be seen from the data on dimensions given above many galls remain atypically elongated for a long time, while others soon adopt a more rounded shape. Both *Peridermium harknessii* and *P. cerebroides* (*?*) are characterized by their more or less spherical to pear-shaped galls which are sharply set off against the unswollen stem. The galls resulting from the inoculations plainly show these characteristics, but the irregularities are far too great to permit the deduction of a rule.

SPORULATION

Not all galls resulting from inoculations reach the stage of aecial production. It is true that the high mortality among the plants used did not permit following each case to its end. But the writer has records of a series with four galls of *Peridermium harknessii* which never produced aecia during the six and a half years they were under observation. The sporulation data for *Peridermium harknessii* are summarized below. They show that the differences in the length of the period from inoculation to spore production do not seem to be induced by the ambient in which the infected plants were kept.

P. sabiniana-P. radiata.	Slit method,	greenhouse,	25, 25, 33	months
P. ponderosa-P. ponderosa.	Spore shower,	nursery,	25, 25, 25	"
P. ponderosa-P. jeffreyi.	"	"	"	24, 37	"
P. ponderosa-P. contorta.	"	"	"	24, 37	"

EFFECT ON THE INFECTED PLANT

Witches'-brooms. The formation of witches'-brooms is a characteristic of both *Peridermium harknessii* and of *P. cerebroides* (*?*). The stimulation of normally dormant axillary buds to vigorous growth is an almost constant corollary of infection and frequently becomes apparent at the time when the stem begins to swell perceptibly. The artificial infections differ in no way from those occurring in the wild state.

Stunting. Both *Peridermium cerebroides* (*?*) and *P. harknessii* have long been known to have a marked retarding effect on the height growth of the infected plants, which is most pronounced when the galls are located on the main stem. The artificially infected pines show the same stunting. Fig. 3 (*Peridermium cerebroides* (*?*), spore shower, *Pinus radiata-P. radiata*) illustrates strikingly the difference in height growth between infected and sound plants. At the beginning of this experiment all four plants were of the same height and in vigorous condition. Of the four plants in one pot, kept in the greenhouse, one escaped infection. Though crowded, it grew in three years and eight months to a height of 239 cm. Two of the infected plants in the same period only reached a length of 67 and 77 cm. respectively. The fourth plant was 61 cm. high when it died.

Killing. The statistics concerning death among the plants used for inoculations are so conflicting that they do not lend themselves to the formulation of a general rule. The writer has still in his possession a pine (*P. radiata-Peridermium cerebroides* (*?*)), the last of a series of 1914 which is now obviously declining. Another plant (*P. radiata-Peridermium cerebroides* (*?*)), inoculated in 1920 and bearing four large galls which completely surround the base of the tree, is still in vigorous condition though it is plainly stunted and its branching system has long lost its regularity.

Cultivation of pines in pots in the greenhouse is by itself likely to produce conditions unfavorable to the health of the plants. Undoubtedly a number of plants would have died even had they not been infected. The interpretation of mortality data of greenhouse plants calls for caution in assigning a definite cause for death.

A few clean-cut series may at least give indications. In April, 1920, eight small seedlings of *Pinus coulteri,* raised from seed in the greenhouse, were inoculated with aeciospores of *Peridermium harknessii* from *Pinus sabiniana.* Of these five developed pronounced galls and witches'-brooms. One died after one year, two after 16 months, the fourth after a year and a half and the fifth survived for over two years. The three uninfected plants were still in thrifty condition after five years. Since all the plants were kept under identical conditions, the cause of death may with safety be assigned to the infection. In another series already referred to (Figs. 1

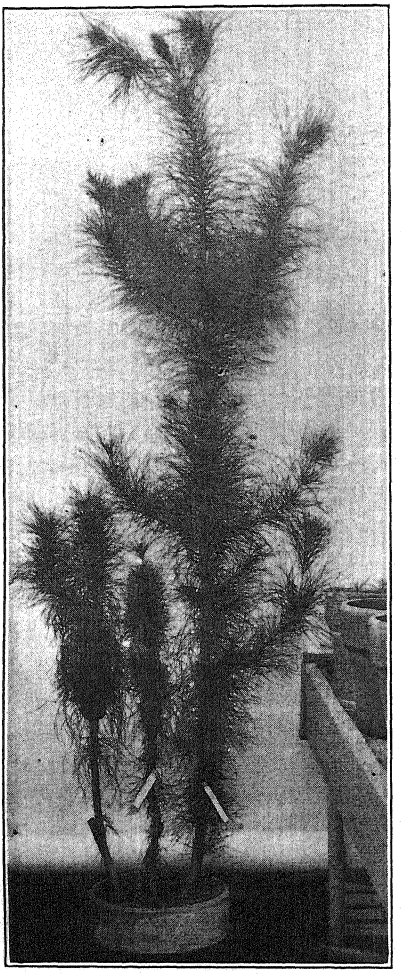


FIG. 3. Stunting of *Pinus radiata* infected with aeciospores of *Peridermium cerebroides* (*?*). One plant which escaped infection has grown to normal height.

and 3, *Peridermium cerebroides* (*?*), *Pinus radiata-P. radiata*) in which four plants in a pot were showered with aeciospores in the greenhouse, one escaped infection. This plant was alive and vigorously growing after six and a half years, while the three infected pines died within three and four years respectively after inoculation. The mortality is far lower in plants grown in the open.

MODE OF INFECTION

The writer had formerly expressed the surmise that infection took place through wounds, most probably through wounds caused by insects. Haack

and Hedgcock both followed the same thought. Klebahn's and the writer's successful experiments with showering unwounded pines with aeciospores seem to prove definitely that wounding is not necessary, while the writer's inoculations with the slit method in which aecial material is directly inserted in the tissues of the stem prove that wounds may serve as a means of entrance. The actual penetration of the germ tube into the host plant in the spore-shower experiments has not yet been observed. Klebahn (7, p. 203) asserts that the youngest shoots offer, without doubt, the principal means of entrance. The writer's results confirm, in the main, this view, but add to this the small spurs or "*Kurztriebe*" which in spore-shower infections seem to be subject in a higher degree to infection than the young shoots. In two series of inoculations (*Peridermium harknessii, P. ponderosa-P. ponderosa,* and *P. ponderosa-P. jeffreyi*) performed in May, the terminal buds of the host plants had sprouted out to a length of 2 to 4 inches. The young shoots were tender and the needle bundles had begun to stretch. One would assume that the young shoots were then in the most favorable condition for infection. Of the 16 plants showered with spores 7 produced 23 galls, all of which originated in spurs near the base of the plants. In no case had a young shoot become infected. In two other series (*Peridermium harknessii, P. ponderosa-P. contorta*) the showering with aeciospores had likewise taken place in May. Eighteen plants were used, of which 13 became infected and produced 72 galls. Of these 15 were located in the top on shoots growing out at the time of the inoculation, while 57 developed in the lower part of the plants, mostly from spurs attached to 2, 3, and even 4-year-old wood.

Klebahn (6, pp. 86–88) had proved that, in *Cronartium ribicola,* infection from sporidia could take place in the needles of *Pinus strobus.* Tubeuf (11) proved experimentally the infection through needles which he was able to follow through to the formation of aecia in the stem. In this country Clinton (2) later reported infection through the needles. It is interesting to note that Tubeuf (11, p. 282) found heavy infection taking place through spurs or *Kurztriebe* near the base of the host plant. He considers the primary leaves and incompletely developed buds, which are not thoroughly protected by bud scales, as particularly subject to infection. Klebahn, Tubeuf, and Clinton have called attention to the yellow spots appearing on the leaves and on the young tender stems as the first indications that the rust mycelium had actually become established.

In direct aecial transmissions the infection apparently does not take place through the needles. Though they were kept under careful observation, the needles of showered plants have never exhibited the characteristic yellow spots nor have such spots been found on the stem. The problem of the mode of entrance is still unsolved. Since Klebahn does not mention yellow spots for his inoculations with *Peridermium pini* it may safely be assumed that none were present.

PYCNIA

As has been reported before by the writer, pycnia have never been found for *Peridermium harknessii* or *P. cerebroides* (?) either in culture or in nature, with the single exception of one gall of *Peridermium harknessii* on *Pinus jeffreyi*. Harkness (5) had already stated that pycnia are unknown on *Pinus radiata, P. ponderosa,* and *P. sabiniana,* the three host plants then known for the mixed species *Peridermium harknessii* Moore. Eight years have elapsed since the writer's last publication on these two Peridermia. Not in a single case has he been able to find pycnia either in the experimentally produced galls or in wild material. This absence of pycnia in the two Pacific Coast forms, one of which is facultatively heteroecious while the other must now in all probability be regarded as autoecious, leaves no basis for speculation as to a possible meaning of the failure to produce pycnia. Artificially induced galls of *Peridermium pini,* which seems to be truly autoecious, produce pycnia, according to Klebahn. It seems likely that, for the two Pacific Coast forms, the production of pycnia is simply repressed but not completely suppressed and that internal pycnia may be found in relatively small numbers.

GERMINATION OF AECIOSPORES

The writer (8) has pointed out that the aeciospores, from either wild or cultivated galls, germinate readily and produce very long germ tubes which are occasionally branched but never show the slightest tendency to produce sterigmata or sporidia. Occasionally one spore sends forth two germ tubes. Dr. B. O. Dodge, in personal communications, has suggested to the writer that this branching may be interpreted in the sense of an abnormal or incomplete promycelium. Fig. 4 illustrates the phenomenon. The branches which frequently are as long as the main germ tube, generally originate near the base of the germ tube, and a septum is generally but not always formed near the angle of branching. In rare cases a second branch may be formed higher up, sometimes also accompanied by the formation of a septum, so that in some cases a two-celled, in others a three-celled structure results. It seems too early to ascribe to these the character of a promycelium. In no case, at any rate, has there been any suggestion of sporidia. The phenomenon is far from being general or even frequent. The fact that it is often totally absent suggests the possibility that it is in itself an abnormality induced by unfavorable moisture conditions in the moist chamber in which the slides with the germinating spores are kept. On the other hand, a septum often appears in the simple, unbranched germ tubes at a distance of two or three times the diameter of the spore. It is too early to speculate on the interpretation of this septum.

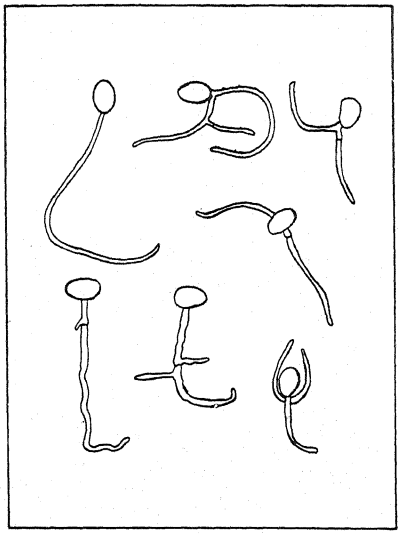


FIG. 4. Aeciospores of *Peridermium cerebroides* (*?*) germinating on a film of water. The germ tube not infrequently has one or two branches, often cut off from the main tube by a septum. Normally the germ tube is single and non-septate or one-septate.

In a former paper the writer has emphasized that the cytology of the mycelium from which the aecium arises, and of the spores formed by the latter, must be given due weight in deciding whether the spore with its germ tube is to be considered as a true aeciospore or as a modified teliospore.

At the writer's request Dr. R. H. Colley and later Dr. B. O. Dodge have had the courtesy to imbed, section, and stain material sent them. In both cases there is not the slightest doubt that the mycelium is uninucleate and that the spores are binucleate. It is difficult to imagine, in the face of these facts, that we are dealing with a teliospore which has lost all the characteristics of a teliospore.

SUMMARY

The gall-forming Peridermium common on *Pinus radiata* and other coastal pine species of California is now believed to be not *Peridermium cerebrum* Peck but a form of unknown relationship, tentatively called *P. cerebroides* (*?*). The gall-forming Peridermium common on *Pinus contorta, P. sabiniana, P. ponderosa,* and other pines of the interior mountain ranges of California is maintained as being the aecial form of *Cronartium harknessii,* nov. comb. This *Peridermium harknessii* has again experimentally been proved to go to Castilleia as the alternate host. The same aeciospore material is, however, equally able to infect pines directly. The author's previous results are confirmed by a large number of experiments, 70 per cent of which were successful for *P. harknessii* when the slit

method is used. With spore-shower inoculation 78.5 per cent were successful in the greenhouse and 44 per cent in the nursery. *Pinus coulteri* has been proved to be susceptible to *Peridermium harknessii* infection. *P. cerebroides* (*?*) also is capable of infecting pines by the spore-shower method. The incubation period for both Peridermia varies considerably. In the experiments reported the periods varied for *P. harknessii* between 3 and 14 months by the slit method and by the shower method from 5 to 27 months. Data are given on the average growth rate for artificially produced galls, on the time of sporulation and on the stunting effect of the infections. Infection apparently takes place not through the needles but through the stem, probably through buds of spurs or *Kurztriebe* poorly protected by bud scales. As in former experiments no pycnia have been found either in artificial or wild galls.

The germination of the aeciospores is discussed. The branching of the germ tube which is not infrequent suggests at least an approach to a promyceliar type. No sporidia have been observed.

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POKKAH-BONG AND TWISTED TOP DISEASES OF SUGAR CANE IN CUBA

C. N. PRIODE[1]

INTRODUCTION

During the last few years a disease of sugar cane, which appears to be identical with the Pokkah-bong disease in Java, has been under observation in Cuba. So far as is known it has not, heretofore, been reported from the Island, although it was observed and recognized in November, 1924, at

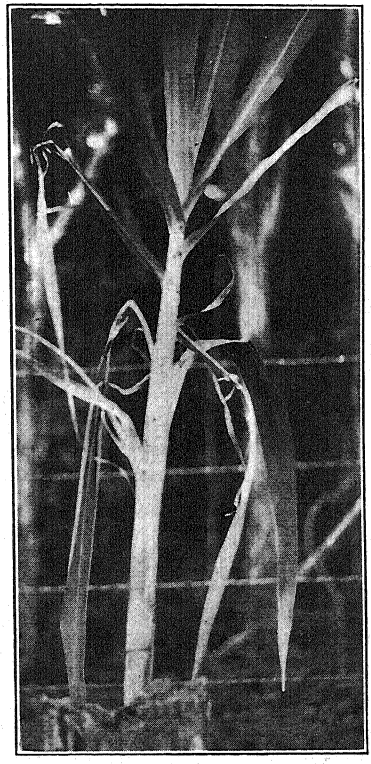


FIG. 1. Cristalina plant affected with Pokkah-bong. Note the splitting and breaking over of the leaves. Photographed Nov., 1924.

Central Santo Tomas, Provincia de Camagüey, by Dr. J. A. Faris, Chief Pathologist of this Station.

Doctor Faris observed a number of Cristalina plants with chlorotic leaf bases and damaged tops. A closer examination revealed the presence of red

[1] Assistant Pathologist, Cuba Sugar Club Experiment Station.

markings on the chlorotic parts. Infection on some of the plants had advanced to the rot stage, causing the death of the whole plant tops. Photographs were made of some of the affected plants, one of which is shown in figure 1. Specimens of the affected leaves were collected and preserved in the herbarium at the Baraguá Laboratory.

Although the disease was not observed until 1924, it probably has been present in the Island for many years. The varieties generally grown in Cuba previous to that time were not particularly susceptible to the attacks of the disease, hence it is quite probable that no serious outbreak ever occurred.

However, with the importation of susceptible new varieties during the last few years, the disease has made itself manifest in several different localities over the Island and, during seasons favorable for its development, it may prove to be of considerable economic importance.

TWISTED TOP

While investigations were being made into the different stages of the above disease, a few plants were observed with peculiarly twisted tops which appeared to be somewhat different from the symptoms of Pokkah-bong. At first, this affection was thought to be a stage of the Pokkah-bong disease but careful examinations indicated it to be a separate and distinct disease, probably identical with the Pokkah-bong or twisted-top disease in Hawaii. The leaves of the affected plants were twisted and tangled but had the color and texture of normal leaves. The twisting of the leaves was the only visible symptom and appeared to be caused by the older leaves clinging together and forcing the younger leaves to grow through them.

This disease probably has existed in the Island since sugar cane was first introduced. Its occurrence on the varieties generally grown in Cuba is so slight that it is of little consequence at present. However, should sensitive varieties be imported, it might, during seasons favorable for its development, prove to be more important. From the following description it will be seen that these two maladies have been reported from other countries as one disease. In a previous report by the writer (6), however, attention was called to the fact that they were separate and distinct from each other.

In 1898 Wakker and Went (7)[2] described and illustrated a disease of sugar cane in Java which they called Pokkah-bong because of the twisted and damaged condition of the affected tops. (Pokkah-bong is a Javanese term which means damaged top shoots.) This was the first report on the disease and very little was known about it at that time. The symptoms as described by them are as follows:

[2] The numbers in parentheses refer to literature cited.

"During the beginning of the rainy season when the cane is young, plants are often found which have a different appearance to the rest of the cane plants in the field. The young partially unfurled leaves show yellow to almost white spots on or near their bases. Rows of holes or cracks usually develop on the white spots. The tips of the leaves are usually green and healthy.

"Sometimes not more than one leaf shows these peculiarities but other cases may be seen where several leaves coming next to each other are infected. Keeping track of these shoots one may observe that the affected leaves do not develop in the usual manner but either break off or split through the affected parts. Dark red to black stripes sometimes develop on the affected leaves. Usually the shoots keep on growing without further damage. Closer examinations of the stalks show them to be somewhat thinner at the points of attachments of the affected leaves. As the disease becomes more severe the internodes under the diseased leaves become funnel shaped, *i.e.*, they are thicker at the tops than at the bases. These internodes are usually very much shorter than the normal ones. Sometimes roots start growing at these short internodes giving the plant somewhat the appearance of a Sereh diseased stick and doubtless this disease has been mistaken for Sereh many times. It is not impossible that both appear at the same time but when it is a case of Pokkah-bong only, the red Sereh stripes do not appear in the joints." (Translation from the Dutch.)

Wakker isolated several species of bacteria but was unable to reproduce the disease from his inoculations with them. The negative results from these inoculations, together with his observations that the disease developed most severely during the warm damp weather which accompanies the change from the dry to the wet season, caused him to conclude that the disease was not of parasitic origin but was the result of the slower-growing outer parts of the leaf funnel, squeezing the rapidly-growing inner parts. This belief was firmly established in Java until the summer of 1927 when Bolle made investigations into the different stages of the disease (1). Observations on the occurrence and spread of infection in the field led to the belief that the disease was of an infectious nature. Bolle's description of the symptoms of natural infection is very similar to that of Wakker and Went, as is seen from the following translation of a recent report (2):

"The first symptoms of Pokkah-bong appear as chlorotic spots on the young leaves, especially near the bases. The appearance of the chlorotic spots is usually followed closely by a malformation of the affected parts. In severe cases the leaves do not unfold normally but are more or less stuck together and are folded, wrinkled, and curled. Red markings frequently appear on the chlorotic parts and these sometimes develop into lens- to rhomb-shaped holes.

"These holes are not easily mistaken for borer holes, as they can easily be distinguished from them partly by the form and partly by the location. The holes caused by borers are circular or round and lead directly through the leaf sheath into the stalk while those caused by Pokkah-bong are as above mentioned: lens- to rhomb-shaped and spread unsystematically over the chlorotic parts, or form rows in the longitudinal direction. The holes formed in the latter way sometimes run together forming long ladder-shaped depressions. The red markings, whether in the shape of holes, spots, or streaks, may continue down into the leaf sheath.

"From the youngest leaves the attack sometimes continues down into the stalk by way of the buds and growing points and dark red streaks may be found extending through several joints. These streaks appear in the joints as fine lines but between the joints they sometimes widen to form long depressions with cross partitions which give them a ladder-shaped appearance.

"The attacks sometimes cause a stunting of the growth by which the internal affected parts can often be recognized, due to the short lengths of the internodes. When the ladder-shaped markings appear at one side close to or at the surface of the internodes the growth is checked only on one side which causes a curved internode.

"When a leaf sheath is strongly attacked a rotting develops on the inside which may pass to the stalk. Here the rot may remain for some time limited to a dry rot of the growing points and the very young internodes. This rotting of the leaf sheath and stalk has often been confused with borer attacks and, where the stalks are badly stunted and the symptoms remain inside the leaf sheath, it is sometimes very difficult to distinguish between the two at first sight. When the stalk is cut lengthwise, however, the difference is very readily seen.

"The above-mentioned symptoms are not always equally developed. Sometimes the deformity of the chlorotic parts is most noticeable, in other cases the red markings are most conspicuous. Also, the different varieties show a great difference both as to severity of attacks and symptoms concerned."

In April, 1927, Edgerton and Tims (3) reported a disease of sugar cane from Louisiana which they believed to be the same as the Java Pokkah-bong. Their description of the symptoms of the disease is short but agrees very well with that of Wakker and Went for the disease in Java. They state as follows:

"A disease identical with or similar to the one known in Java as Pokkah-bong, a Javanese term meaning twisted top, has in recent years made its appearance in Louisiana. The identity of the Louisiana disease with the one in Java was confirmed by H. Atherton Lee, of the Hawaiian Sugar

Planters Experiment Station, while making a trip through Louisiana in the summer of 1926. While the disease was very common during the summers of 1925 and 1926, as far as is known, this is the first published report on it.

"Pokkah-bong first shows in the new leaves as they come out of the terminal bud. The leaves are light colored and are much twisted, frequently to such an extent that they are practically tied together. Sometimes these young leaves rot and, on this account, the disease has frequently been called top rot in Louisiana. Usually, however, the leaves grow partially out of this twisted condition. New leaves that develop later may show little signs of the disease.

"The internodes which form in association with the twisted leaves very frequently remain short. The disease was first observed on the newly introduced Java seedlings, but later appeared on the Purple, D 74, and other Louisiana varieties."

In January, 1928, Lee (5) reported a Pokkah-bong or twisted-top disease from Hawaii, which he states is a non-infectious disease caused by the friction of the leaf hairs which prevents the leaves from unfolding normally. He writes as follows:

"One notices that some of the tall cane stalks appearing above the usual height of the cane fields do not have their leaves expanded normally but a mass of closely wrapped, twisted ribbons of leaves. Examining more closely one will observe that many of the leaves are split and the growing mass of the cane tops is wrapped around by such split leaves. The outermost leaves of the bunched cane tops are of the usual green color of normal leaves but the younger, inner leaves are of a greenish-yellow color. Moreover these younger, inner leaves are not of the usual texture of normal leaves but are buckled and corrugated and have somewhat the character of crumpled cabbage leaves. These inner leaves are very rigid and seem to be under considerable mechanical tension. Less advanced stages of the disease are sometimes seen in which the whole tops of the plants are not tied up but the tips of the leaves as they emerge from the growing point do not separate from each other. Such cases with the lower parts of the leaf blades distended but tied together at their tips have an arched appearance."

It seems evident from the above reports that there are two separate and distinct diseases described under the name Pokkah-bong. The Pokkah-bong diseases in Java, reported first by Wakker and Went and later by Bolle, probably are identical. The symptoms described in each report are very similar and the colored plates given by these workers portray stages of the disease which strongly suggest a parasitic origin. It is also quite probable that the trouble reported from Louisiana is of parasitic nature and identical with the disease in Java.

The Pokkah-bong or twisted-top disease reported by Lee from Hawaii, however, appears to be a distinctly separate malady. The symptoms described do not suggest a parasitic origin but, as shown by Lee, indicate the

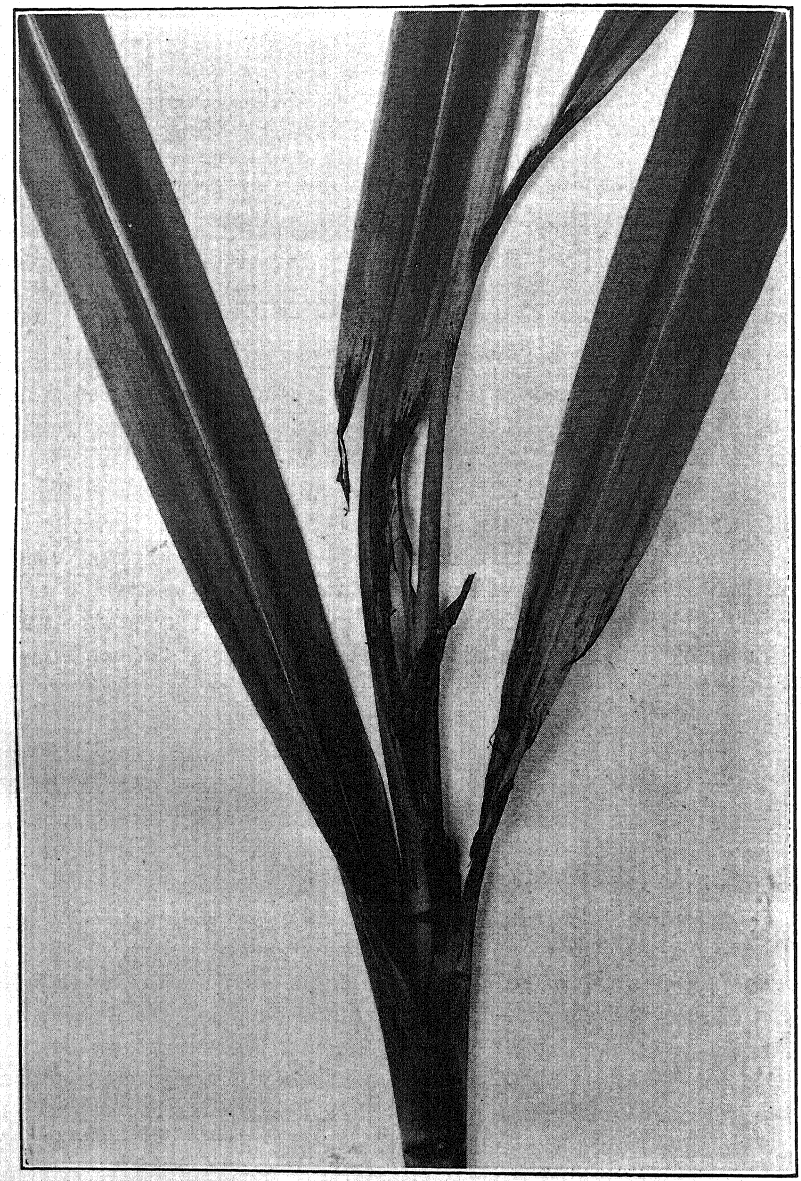


FIG. 2. Pokkah-bong infection on B H 10/12 plant, showing ragged appearance of leaf bases caused by the falling out of the rotted areas after they became dry.

twisting of the tops to be caused by a simple mechanical friction of the leaves which prevents their normal separation. This also is true of the twisted-top disease in Cuba. The symptoms of Pokkah-bong in Cuba, however, are very similar to those of the Java disease, as the following description will show.

DESCRIPTION OF THE POKKAH-BONG DISEASE IN CUBA

The first symptoms of Pokkah-bong usually appear as chlorotic spots on the bases of the young leaves. These spots become apparent as the leaves emerge from the leaf spindle. Infection appears to take place in the young leaves of the spindle, probably while it is full of water.

In cases of light infection the chlorotic spots are the only symptoms. Usually, however, red markings develop on the chlorotic parts before the leaves are fully unrolled and, when the attack is very severe, may result in a wet rot. After the leaves separate from the leaf roll the red markings may dry and fall out, giving the leaf the ragged appearance seen in figure 2.

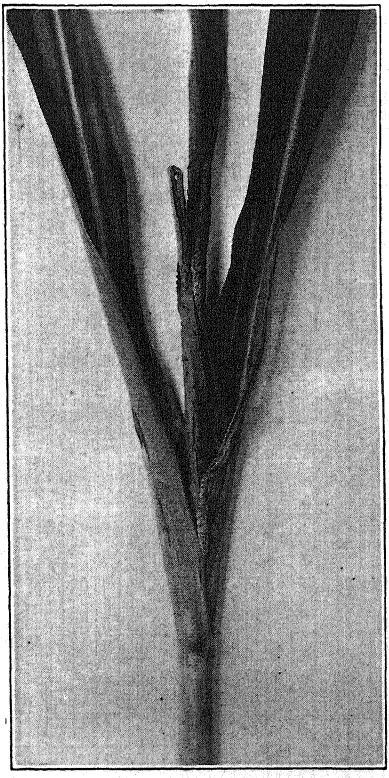


FIG. 3. Plant affected with Pokkah-bong, showing how the old chlorotic leaves become corrugated and drawn, forcing the younger leaves to buckle and split their way through to the outside.

Sometimes such leaves break over and fall off. Where the infection reaches down into the very young tissues of the leaf-roll, a rot sometimes develops which may destroy the whole leaf spindle down to the growing point. Usually, however, the younger, inner leaves grow through the affected parts without being seriously damaged.

Some cases have been observed where the chlorotic parts of the outside leaves become wrinkled and drawn and do not unfold normally but cling tightly together, forcing the rapidly growing, younger leaves to buckle and split their way through to the outside, as in figure 3.

In very severe cases the leaves become so wrinkled, corrugated, and drawn that they cannot unfold but split and twist around each other, forming a closely wrapped mass of the whole plant tops. Figure 4.

On susceptible varieties infection is not limited to the leaves but may pass from the leaves and leaf sheaths down into the stalks. When the stalks are split open, red streaks can be seen extending from the points of attach-

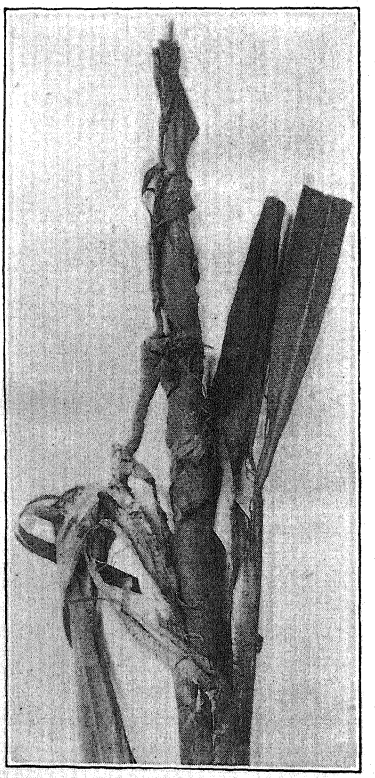


FIG. 4. A severe case of Pokkah-bong on a POJ 2714 plant. The corrugated and drawn leaves have tied the top into a closely wrapped mass. Note the ragged appearance of the older leaves where the red markings have fallen out.



FIG. 5. Section of a POJ 2878 stalk infected with Pokkah-bong, showing the surface cavities. When such stalks are split open red lines or streaks extending down into the stalk can usually be seen.

ment of the affected leaves far up into the stalks. These red lines sometimes widen between the joints to form long sunken cavities, which usually cannot be detected until the stalk is split open. Sometimes, however, they are evidenced by the short lengths and bulgings of the affected internodes.

In some cases the infection does not penerate very far into the stalk but is confined to the surface tissues. Here, the cavities may be observed extending from the base of an infected sheath, up the outside of the stalk. A section of an infected stalk showing the surface cavities is shown in figure 5.

Sometimes the cavities develop on the part of the stalk enclosed by the leaf sheath and may pass unobserved until the sheath is removed. Their presence can usually be detected, however, by the curved internodes which result from the uneven growth on the sides of the infected internodes.

Any or all of the above symptoms may occur on the same plant. The chlorotic spots on the leaves, however, are usually the first and most conspicuous symptoms by which the disease may be recognized. These symptoms of Pokkah-bong are very clearly illustrated by the colored plate (Plate I) in this paper. The disease usually makes its appearance shortly after the beginning of the spring rains and continues its attacks throughout the rainy season. During dry periods it does not appear to be of much consequence.

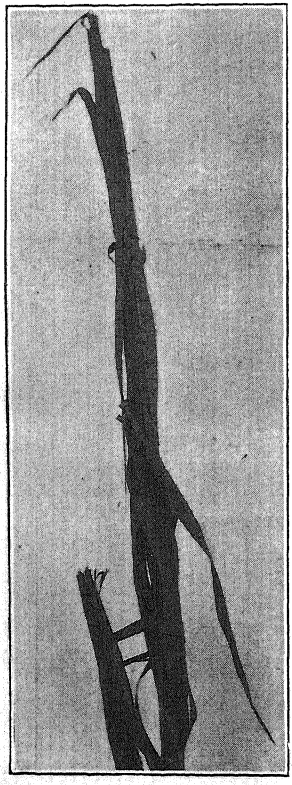


FIG. 6. A plant affected with twisted top, showing how the young leaves are held together by the split older leaves.

DESCRIPTION OF TWISTED TOP IN CUBA

Twisted top, as it occurs in Cuba, affects only the plant tops. The older leaves appear to be held together by some mechanical means and do not separate normally. The growth of the younger leaves from below usually causes the older leaves to split and tangle. These in turn have a tendency to wrap around the young leaves and the whole plant top may grow into a tangled mass of split leaves. Sometimes the younger leaves of the leaf-roll grow through the split, older leaves but are so closely wrapped by them that they do not unfold but grow into a slender spike-like mass of wrapped leaves, as in figure 6.

Other cases may be seen where the spindle of the plant is not wrapped around by the older leaves but the tips of the leaves cling together and do not separate. As the growth continues from below, the leaves form bows or arches. In some cases these arches begin to form when the plant is young and, as each new leaf emerges from the leaf-roll, its tip becomes enmeshed

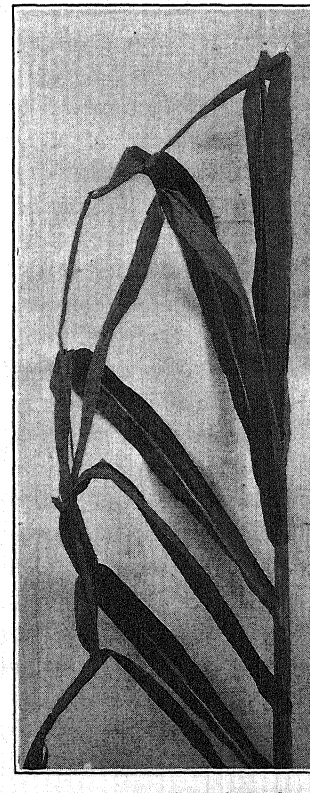


FIG. 7. Twisted top, showing how the tips of the leaves become entangled, forming arches as the growth continues.

with the tips of the older leaves and an additional arch is formed. This process may continue until all the leaves on the plant will be formed into a series of arches, one over the other, on one side of the plant. Figure 7.

No parasitic symptoms were observed on any of the plants affected with twisted top. The color and texture of the affected leaves were the same as for normal leaves.

Unlike Pokkah-bong, the disease occurs mostly during the dry seasons; only a few scattered cases may be seen during the rainy periods.

The dissimilarity of the above symptoms of the two diseases indicates Pokkah-bong to be a disease separate and distinct from twisted top. The results obtained from the following experiments, however, will make this fact more evident and more clearly understood.

EXPERIMENTS WITH THE POKKAH-BONG DISEASE IN JAVA

Bolle's observations on the occurrence and spread of infection in the field led her to believe that the Pokkah-bong disease in Java was of parasitic nature. Accordingly, she made several isolations from the discolored vascular bundles in stalks affected with Pokkah-bong. In nearly all her cultures she obtained the same Fusarium later identified by Wollenweber as *Fusarium moniliforme* Sheldon. Other species of fungi and several bacteria also were isolated but none of these proved infectious when inoculated onto healthy plants. Inoculations made from pure cultures of the Fusarium fungus, however, produced typical symptoms identical to those she observed on naturally infected plants in the field.

On one set of plants the inoculum—a suspension of spores in distilled water—was dropped into the leaf funnel from a pipette. Only leaf symptoms developed from these inoculations. Chlorotic spots and red markings soon appeared on the bases of the young leaves but no stalk symptoms were observed. On the other set of plants the inoculum was injected into the heart of the leaf-roll, just above the vegetative point, by means of an injection syringe. By this method the spores were put in direct contact with the young, tender tissues of the leaf funnel and, from these inoculations, both leaf and stalk symptoms developed. The chlorotic spots and red markings soon made their appearance on the bases of the leaves and from these the red lines could be seen extending down into the stalk. In some cases the red lines widened between the joints to form the ladder-shaped figures or cavities characteristic of the advanced stages of stalk infection. On some of the plants, infection resulted in severe cases of top rot, in which the whole of the leaf rolled and the growing point died.

About two and one-half weeks after the inoculations were made the same Fusarium was reisolated from infected tissues far above the point of injection. Inoculations made from the reisolated cultures produced typical Pok-

kah-bong symptoms. These inoculation experiments were repeated several times on different varieties of cane with similar results.

EXPERIMENTS WITH THE POKKAH-BONG OR TWISTED-TOP DISEASE IN HAWAII

In his experiments with the Pokkah-bong disease, in Hawaii, Lee mentions no attempt to isolate any causal organism. His observations on the occurrence of the disease in the field, together with a remark by Mr. Norman King, agriculturist for the Pioneer Mill Company, that there seemed to be some cause of friction as the leaves were emerging from the central cylinders of the cane tops and that this friction caused a buckling of the rapidly growing young leaves, led him to believe that the trouble could be produced artificially by increasing the friction on the young leaves. This belief was strengthened by a previous study of leaf structures by Lee in which he observed the presence of short, rigid hairs or bristles on the leaves of H 109 plants. (H 109 was the variety most severely affected by the disease.)

To test this theory Lee selected several plants of the H 109 variety and bound the leaf spindles with ordinary black friction tape. The purpose of this was to increase the friction on the rapidly growing young leaves. A fine reproduction of twisted tops developed from this increased friction on nearly all the plants so treated. The leaves became corrugated and enmeshed at their tips, presenting an arched appearance as they emerged, and they could not separate. The next emerging leaves were obstructed by the arched entanglements of the older leaves and were forced to grow through them, causing them to split and twist. The split, older leaves then had a tendency to tie up and increase the friction on the younger leaves so that the whole top growth usually resulted in a closely wrapped mass of split leaves.

In another experiment he produced typical cases of twisted tops by abruptly bending the leaf spindles about middle ways of their heights. These experiments were repeated with like results in each case. Lee makes no mention of the parasitic symptoms characteristic of the Pokkah-bong disease in Java. The twisting and tangling of the leaves appeared to result from the leaf hairs clinging together and preventing the normal separation of the leaves.

In concluding his report Lee makes two statements, as follows:

(1) "It seems evident that twisted top as it occurs in Hawaii and probably in Louisiana, is a mechanical difficulty and non-infectious."

(2) "The writer at first inclined toward the feeling that the Java and Philipppine form of the disease was infectious and distinct from this form common in Hawaii. With the development of this work, however, one begins to feel that the form in Java and the Philippines might simply be a more severe form of this mechanical injury. One would at least feel doubtful in considering any form of this disease as infectious."

It is evident from the results of these experiments by Lee that the twisted-top disease in Hawaii is a non-infectious disease caused by the mechanical friction of the leaf hairs, as described in his report. It is also evident from his conclusions, that Lee considers the disease to be the same as, or a different stage of, the Pokkah-bong disease in Java. These widely different experimental results obtained by Bolle in Java and Lee in Hawaii seem to indicate that they were dealing with two separate and distinct maladies under the same name. To determine whether this was the case the following experiments were undertaken:

EXPERIMENTS WITH THE POKKAH-BONG DISEASE IN CUBA

The experiments with the disease in Cuba were performed at this station and were carried out as follows:

Experiment 1.—One row of the seedling variety of Baraguá 2 was selected for the experiment. All the plants in the row were closely examined but no evidence of the disease was observed. This variety does not easily become infected with Pokkah-bong under natural field conditions. Six plants of one stool were inoculated with Fusarium spores from a pure culture isolated from the red lines deep down in a POJ 2878 stalk affected with Pokkah-bong. A suspension of the spores in distilled water was injected into the center of the leaf funnels, just above the growing points, by means of an injection syringe.

Six other plants of the same stool were inoculated in the same way, except that distilled water alone was used for the inoculum. These latter plants, together with the uninoculated plants in the row, served as checks. About two weeks after date of inoculation the plants were examined and five of the six stalks showed typical symptoms of Pokkah-bong infection. These symptoms appeared strikingly similar to those described by Bolle from inoculations with the Fusarium spores in Java. One of these plants is shown in figure 8.

The chlorotic spots on the leaves were the first symptoms to appear. These were followed very closely by the red markings. Infection in two of the plants had advanced to the top-rot stage and the leaf-rolls of these plants were completely dead. No stalk symptoms developed on any of these plants.

None of the checks inoculated with distilled water alone showed any symptoms of infection and none of the untreated plants in the row were infected, although plants of other varieties in adjoining rows were severely infected.

Experiment 2.—The above experiments were repeated on other varieties of cane. The same Fusarium fungus was reisolated from the plants artificially infected in the first experiment. Spores from these new isolations were injected into a few plants of each of the following varieties: FC 306;

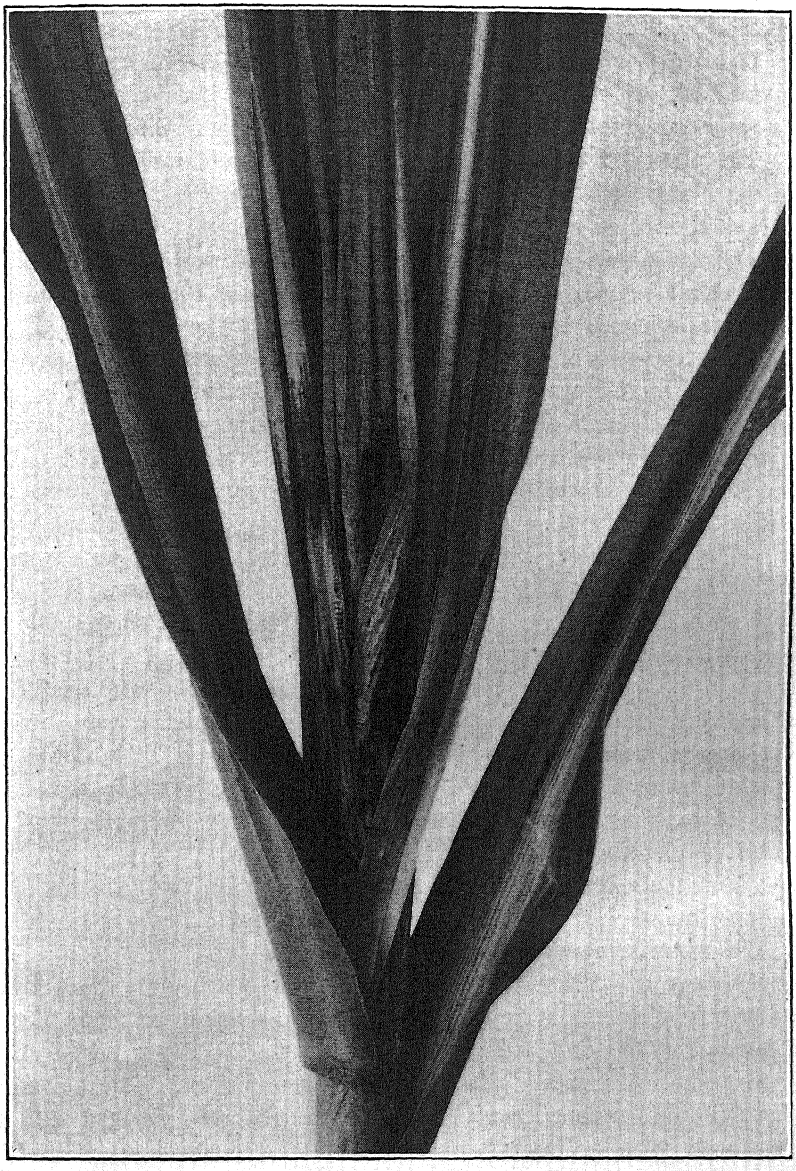


FIG. 8. Pokkah-bong produced from inoculations with Fusarium spores. Note the markings on the chlorotic parts.

BH 10/12; D 109; SC 12/4; PR 525; and Cristalina. Checks were kept for each of the varieties except PR 525. All the varieties inoculated showed symptoms of Pokkah-bong infection within ten days from date of inoculation, though not all the plants of each variety became infected.

The red markings and chlorotic spots were very pronounced on all the plants infected. One plant of FC 306, one of BH 10/12, one of SC 12/4 and two of PR 525 showed severe cases of top rot. The leaf rolls of these plants were completely dead. None of the check plants showed any infection. The plants in this experiment were in pots and were kept in cages inside the plant house where the possibility of infection from wind-borne spores was greatly reduced.

Experiment 3.—Observations on the natural occurrence of the infectious type of Pokkah-bong in the field showed that none of the varieties used in the above experiments is particularly susceptible to the attacks of the disease. Very few cases of natural infection were observed on these varieties. The variety POJ 2878 was reported from Java to be very susceptible to the disease. But no plants of this variety were available at the time the above experiments were made. However, fourteen plants were secured later and seven were inoculated with Fusarium spores on August 8. The spores were injected into the leaf-rolls with the injection syringe, as in the above experiments. The other seven plants were used as checks. The plants were examined on August 20. All of those inoculated with the spores were severely infected with Pokkah-bong. The bases of the leaves showed the chlorotic spots and red markings very plainly. Infection on four of the plants had advanced to the top-rot stage and on these the spindle rolls were dead down to the growing points. Figure 9. None of the check plants were infected.

The results from these inoculation experiments leave little doubt that the Pokkah-bong disease in Cuba is infectious. However, to remove all possible chances of outside infection, inoculations were made with the Fusarium spores under absolutely aseptic conditions.

The same method was employed as that devised by Faris for his inoculation experiments with the brown-stripe and eye-spot diseases of sugar cane (4). The experiment was performed as follows:

Experiment 4.—Fifteen glass cylinders, 50 centimeters high by 10 centimeters in diameter, were filled with sand to a height of from 15 to 20 centimeters. (The sand had previously been washed for a period of six hours to remove the excess salts.) It was then watered with a chemical solution suitable for cane growing. The cylinders were plugged with cotton, wrapped with ordinary wrapping paper, and sterilized in a large autoclave under 25 lbs. pressure for five hours. Smaller cylinders also were filled with sand, wrapped, and sterilized with the larger cylinders.

Single-bud seed pieces of the variety POJ 2727 were soaked in a 1/1000 solution of mercuric bichloride for 15 minutes. They were then transferred directly to the large cylinders and covered with the sand from the small tubes. The cotton plugs were replaced and the tops of the jars rewrapped

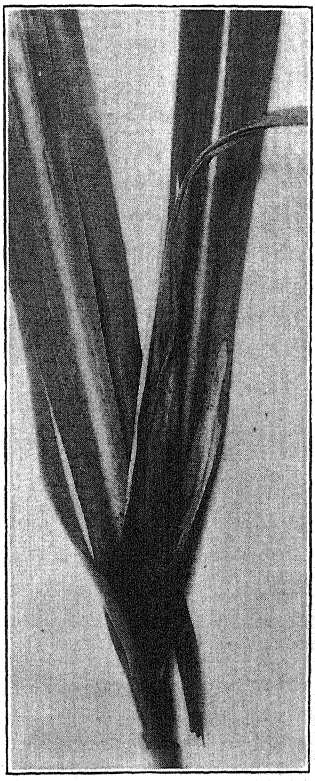


FIG. 9. Pokkah-bong produced by injecting the Fusarium spores into the heart of the leaf-roll. The central stem is completely dead.

with paper. The whole procedure of planting the cane in the jars was done in an aseptic inoculating chamber.

When the cane had germinated and the plants had reached a height of from eight to ten inches, they were inoculated with spores from a pure culture of the Fusarium fungus, as follows: The jars were placed in the aseptic inoculating chamber and the cotton plugs removed just enough to allow the insertion of an atomizer tube. Twelve of the plants were then thoroughly sprayed with a suspension of the spores in distilled water, the cotton plugs replaced and the tops again wrapped with paper. The three uninoculated plants served as checks.

About 15 days after date of inoculation there were no definite symptoms of Pokkah-bong on these plants. There were faint chlorotic spots on the leaves of some of the plants but these could not be taken as definite Pokkah-bong symptoms.

The jars were again brought into the inoculating chamber and the plants were inoculated a second time. This time the spores were injected into the young tissues of the leaf-rolls by means of the injection syringe.

Twelve days after inoculations were made, three of the plants showed symptoms of infection. After fifteen days, two of these plants had developed severe cases of top rot and the spindle rolls were completely dead. The other plants together with the checks remained free of the disease.

These results prove definitely that the Pokkah-bong disease in Cuba is infectious and caused by a species of Fusarium. The symptoms produced from the above inoculations were identical to those described by Bolle from her inoculations with the Fusarium spores in Java and leave little doubt that the two diseases are identical.

EXPERIMENTS WITH TWISTED-TOP DISEASE IN CUBA

In these experiments no attempt was made to isolate any causal organism. No parasitic symptoms were observed on any of the plants in the field, so the disease was not believed to be of parasitic origin but closely related to, if not identical with, the twisted-top disease in Hawaii. The experiments were performed as follows:

Experiment 1.—Six plants in each of two stools of the variety Baraguá 2 were selected for the experimental work. The spindles of the plants in stool No. 1 were bound near the base with ordinary black friction tape. The spindles of the plants in stool No. 2 were bent abruptly about middle ways of their heights. About three weeks later all of the plants with the wrapped spindles, and all with the bent spindles, showed varying degrees of twisting. On some of the plants the tops were intricately tied up. The older leaves had a tendency to cling together and were split by the growth of the younger leaves from below. The younger leaves were then wrapped around by the split, older leaves and the whole top growth usually resulted in a mass of split and tangled leaves. Figure 10.

In most of the cases where the spindles were wrapped with the friction tape the leaves did not split or tangle but the tips were held together, forming arches similar to those described by Lee (5). As the new leaves emerged from the leaf spindle their tips became enmeshed with the tips of the older leaves, and additional arches were formed, all on one side of the plant. In some cases the tips of the leaves died back to the point where the tape was wrapped around them. Figure 11.

There were no parasitic symptoms connected with these, however, and the dying back appeared to be caused by the tight wrapping of the tape. No chlorosis nor red markings were observed on any of these plants nor were there any indications of top rot.

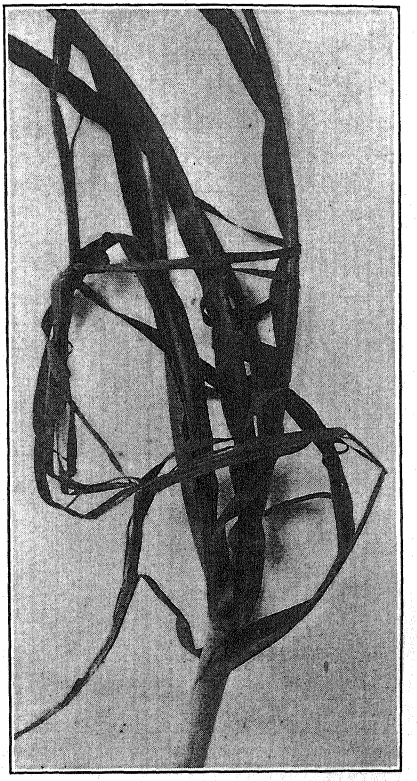


FIG. 10. Twisted top produced by the abrupt bending of the leaf spindle when the plant was young. Note the ribbon-like strips of the split, older leaves.

In another experiment ten plants of the seedling variety Baraguá 10 were selected. The leaf spindles of all ten plants were bent abruptly about middle ways of their heights. About two weeks later all of these plants had developed typical cases of twisted tops. The bending of the leaves appeared to hold the leaves together in such a way that the hairs enmeshed and would not separate. The older leaves, becoming entangled, obstructed the growth of the younger leaves. These in turn either split the older leaves and grew through them or became entangled with them and formed the arches described above.

The results of these experiments confirm the work of Lee and prove that the twisted-top disease in Cuba is non-infectious and identical with the twisted-top disease in Hawaii.

POKKAH-BONG AND TWISTED TOP SEPARATE AND DISTINCT DISEASES

The results of the above experiments also prove Pokkah-bong and twisted top to be separate and distinct diseases. Each has its own distinctive symp-

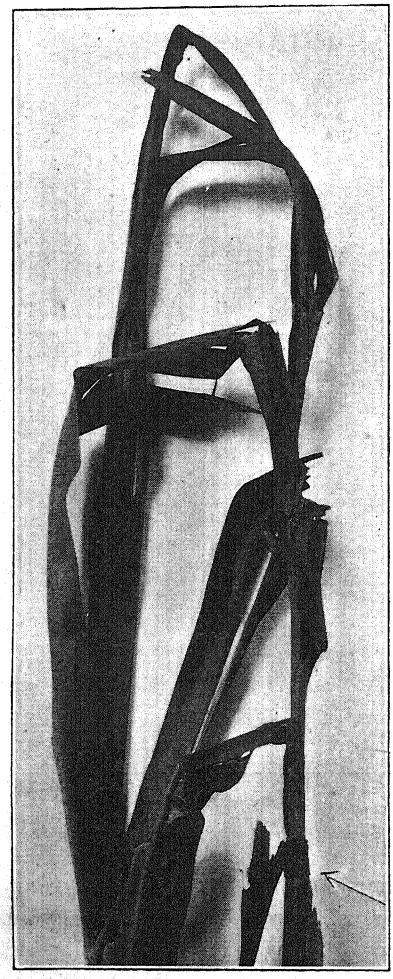


FIG. 11. Twisted top produced by wrapping the young leaf spindle with friction tape. The tape can be seen at the bend of the first leaf. The tips are dead back to this point.

toms which readily distinguish it from the other. The following comparison of these symptoms will make this fact more evident and more clearly understood:

Symptoms of Pokkah-bong

1. Affected tops may become twisted, corrugated, and drawn.

2. Bases of affected leaves usually chlorotic, with red markings.

Symptoms of twisted top

1. Top leaves split, twist, and tangle, but the texture remains the same.

2. Color of affected leaves same as for normal leaves.

Symptoms of Pokkah-bong	*Symptoms of twisted top*
3. Leaf sheaths may show red markings, with cavities or holes.	3. No symptoms observed on leaf sheaths.
4. Top rot sometimes develops which destroys the whole leaf spindle.	4. No indications of top rot.
5. Red streaks and cavities may develop on and in the stalks of affected plants.	5. No stalk symptoms observed.
6. Attacks most severe during rainy periods.	6. Affection most severe during extremely dry periods.
7. Caused by species of Fusarium.	7. Caused by simple mechanical friction of the leaves.

The above comparison shows that the only distinguishing symptom of the twisted-top disease is the twisting and tangling of the affected leaves. No sheath or stalk symptoms were observed. The symptoms of Pokkah-bong, however, are numerous. The leaves, leaf sheaths, and stalks may all become infected and show the parasitic symptoms.

The only symptom common to both diseases is the twisting or damaging of the plant tops. This one symptom, due to the fact that the diseases have been but vaguely understood, led to the belief that they were the same or different stages of the same disease. Where the two maladies occur on adjoining plants, as was the case in some of the experiments performed by the writer, their differences can readily be seen. Each has its own distinctive type of twisting and one can readily be distinguished from the other.

DISTINCT NAMES SUGGESTED FOR THE DISEASES

The name Pokkah-bong, as stated in another part of this paper, was given to the disease in Java by Wakker and Went, in 1898. Since that time numerous reports of Pokkah-bong diseases have been published from many parts of the world. These, for the most part, have been but vaguely understood and we have no assurance that they were the same in each case as the Pokkah-bong disease described and illustrated by Wakker and Went. It is now known that the twisted-top disease in Cuba, which appears to be identical with the Pokkah-bong disease reported from Hawaii, is a trouble separate and distinct from the infectious Pokkah-bong and it is quite probable that some of the other diseases reported as Pokkah-bong are of like nature.

Where the diseases occur in mild form in a field, an observer, not knowing the existence of the two diseases and not being familiar with the characteristic symptoms of each, would perhaps mistake them to be the same or different stages of the same disease. Now that they have been proved to be two separate and distinct maladies it is necessary that each be given a specific name, so that they can be recognized and distinguished from each other.

Since the term Pokkah-bong was first given to the malady in Java, it is the opinion of the writer that this name should be given exclusively to that disease and another name be selected for the non-infectious disease.

The disease in Hawaii has been called "Topknot," "Twisted Top" and various other names descriptive of the twisted and tangled condition of the affected tops, and since this twisting is the most conspicuous symptom of the disease the term twisted top naturally suggests itself as the most appropriate name for the disease.

The adoption of these names will probably clear away the confusion which has heretofore surrounded these two maladies and will serve to distinguish one from the other when they are observed in the field.

VARIETAL RESPONSES TO THE DISEASES.

Pokkah-bong

The varieties of cane commercially grown in Cuba at the present time appear to differ greatly in their susceptibilities to the attacks of Pokkah-bong. Most all of them are susceptible to a certain extent, but on some varieties the symptoms of the disease are barely noticeable.

The Cristalina and D 109 varieties contract the disease very readily but infection is usually confined to the leaves. The chlorotic spots and red markings may be very pronounced on the leaves and a few cases of top rot may develop during favorable weather but the loss from the disease on these varieties is very small. No indications of stalk symptoms have been observed on either of these varieties, though they might develop under more favorable conditions.

The varieties SC 12/4, FC 306, BH 10/12, Yellow Caledonia and some of the Porto Rican seedling canes also are somewhat susceptible but the attacks on these varieties are usually very light, with little resulting damage.

Most of the newly introduced Java canes are more or less susceptible to the disease. Some of these show only faint, chlorotic symptoms on the bases of the leaves; while on others, the attacks are more severe. The POJ 2878 variety appears to be most severely infected of any of the varieties so far observed in Cuba. In a recent report by Bolle (2), POJ 2722 and POJ 2878 are given as the most susceptible varieties in Java.

On the experiment plots of this station, where the overhead method of irrigation is employed, about 60 per cent of the plants of the POJ 2878 variety became infected. The regular overhead sprinkling of the plants kept the tops moist and made conditions especially favorable for the development of the disease. Some of these plants showed only the chlorotic leaf symptoms, while on others the infection had advanced to the top-rot stage,

killing the leaf-rolls and growing points. The loss on these canes from top rot, however, was very small. On a few plants of this variety infection had extended down into the stalks, forming the cavities described and illustrated in another part of this paper. Under ordinary field conditions the percentage of infection on this variety probably would be very much less than was observed on the irrigated plots.

The POJ varieties 2727, 2714, and 2883 are also susceptible to the disease but to a much less degree than POJ 2878. Infection on POJ 2725 was observed to be very slight.

Twisted top

Twisted top does not seem to be limited to any particular variety but develops on most any plant where the friction of the leaves is increased. The few cases of natural occurrence in the field, observed during the past summer, were widely scattered and, in nearly every case, resulted from some injury to the plant tops.

SUMMARY

The symptoms of Pokkah-bong and twisted-top diseases are described and illustrated. Experiments were performed which proved them to be separate and distinct diseases.

Pokkah-bong is shown to be an infectious disease, probably identical with the Pokkah-bong disease in Java. Experiments proved it to be caused by a species of Fusarium, very similar to *Fusarium moniliforme* Sheldon described as the organism causing the Pokkah-bong disease in Java.

Twisted top is a non-infectious disease probably identical with the Pokkah-bong disease reported from Hawaii. It is caused by a simple mechanical friction of the leaves.

Most of the commercially-grown varieties of cane in Cuba are shown to be susceptible to the attacks of the Pokkah-bong disease but the consequent loss is small. The POJ 2878 variety appears to be the most severely affected of any of the varieties under observation.

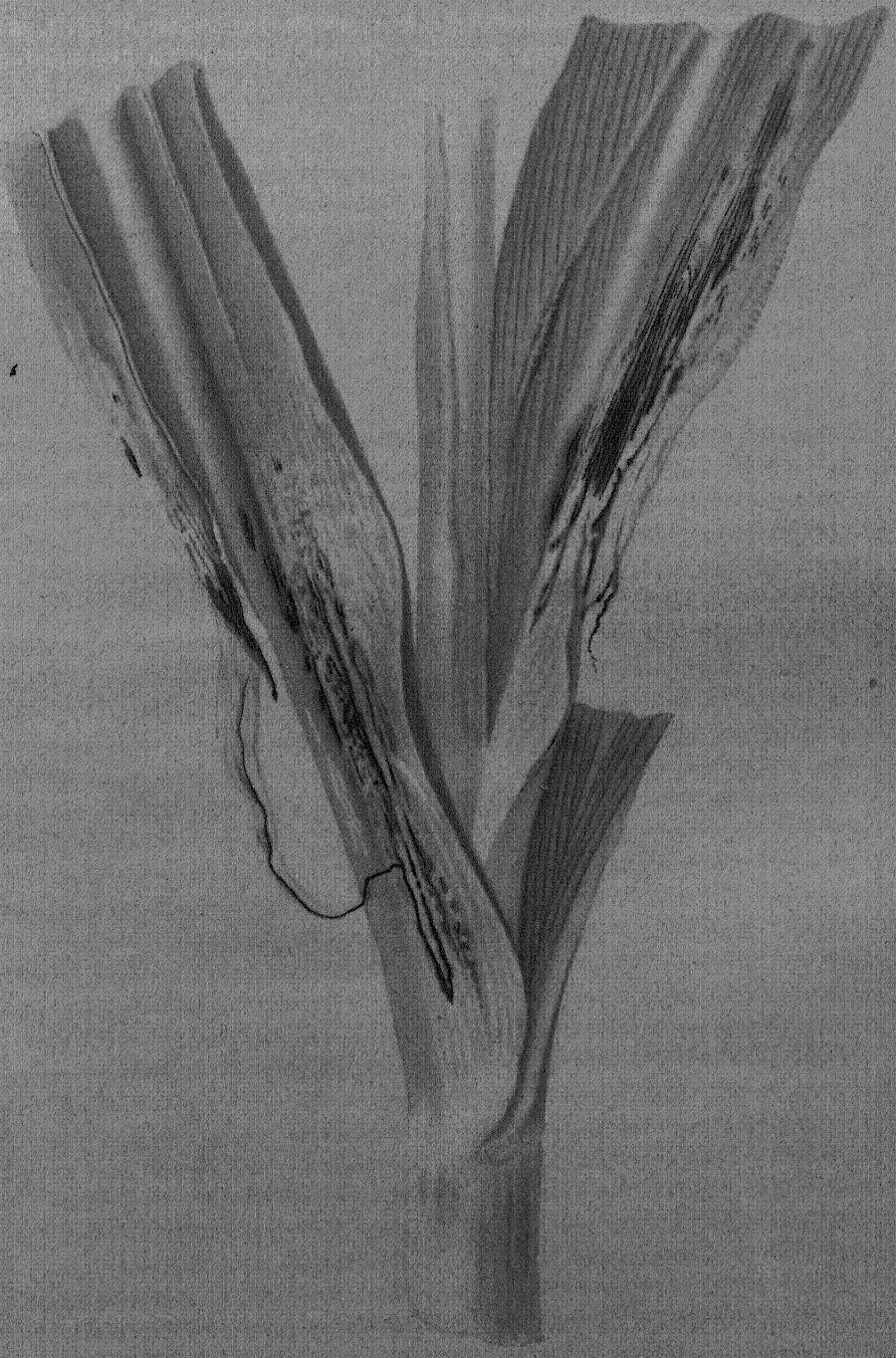
The writer wishes to express his appreciation to Dr. J. A. Faris for valuable suggestions and advice during the progress of this work and for a careful reading and criticism of the manuscript.

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PLATE 1.



RHIZIDIOCYSTIS ANANASI SIDERIS, NOV. GEN. ET SP., A ROOT HAIR PARASITE OF PINEAPPLES[1]

C. P. SIDERIS

INTRODUCTION

Rhizidiocystis ananasi is one of the many organisms that infest pineapple roots and are more or less responsible for the development of the disease known as "pineapple wilt." This organism is apparently new to science, since no mention of it can be found in the available literature.

The organism attacks and kills the root hairs of pineapple plants. It does not invade any of the other exodermal cells or the interior tissues of the root. Parasitism is definitely restricted to the root hairs proper. One may find the mycelium covering the very tip of the root but, even in such cases, the organism has never been observed to invade other cells except those of young and tender root hairs that are still in the process of development in the region of the root tip. There is no rot associated with infected tissues; and the death of the main root tissues, which sometimes occurs, is due to secondary causes.

TECHNIQUE

Owing to the fact that the organism is a strict parasite and not culturable on synthetic media, its isolation from infected fields required a special technique. Repeated attempts to grow it on sterilized pineapple roots that had been grown in water cultures and all of whose root hairs were intact, but dead, failed; the only recourse left was to grow it on living root hairs and study it there. The best method for conducting such studies was found to be by means of root-study boxes. Such boxes were used quite extensively by Dr. J. F. Illingworth, of the Entomology Department, Experiment Station of the Association of Hawaiian Pineapple Canners, and the organism was first observed in one of his boxes. These root-study boxes, known otherwise as root cages, were first introduced by Comstock (4) and later were used by Illingworth (8) for the study of the behavior of insects in the soil. The original plans for the boxes have been considerably modified by various members of this Station staff to meet the requirements of specific problems. Some of the boxes are illustrated in figures 1, 2, and 3. They may be made in any size for specific purposes, but the size most often used for these studies measures $16 \times 14 \times 2\frac{1}{2}$ inches. The essential feature is to have two glass sides through which the growth and development of the roots of the plant growing therein may be observed microscopically without the least

[1] Technical paper No. 6 of the Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii.

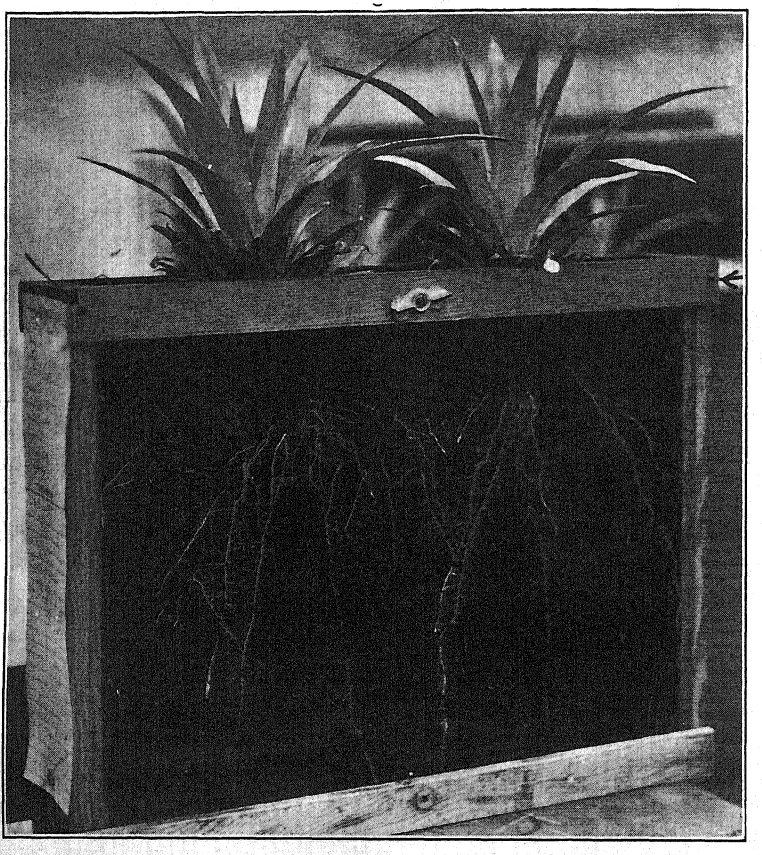


FIG. 1. A Comstock type root study box. In the more recent models hooks are being used in the place of the screws marked with an arrow. The wooden panel that is placed over the glass to keep the sunlight out has been removed in this case to show the roots as they appear against the glass side.

disturbance to the plant. Except during observation periods the glass sides are covered with wooden blinds. Both animal and vegetable organisms that live near or directly on the roots may likewise be observed and their relationships studied. The boxes are so constructed that the glass sides can be removed by releasing a hook without greatly disturbing the soil or the roots of the growing plant. Frequent microscopic examinations also are possible, as well as removal of diseased roots without injury to nearby roots or disturbance of the soil around them.

ISOLATION OF THE FUNGUS

The method used for the isolation of the fungus from various field soils was as follows: The soil under consideration was placed in a root-study

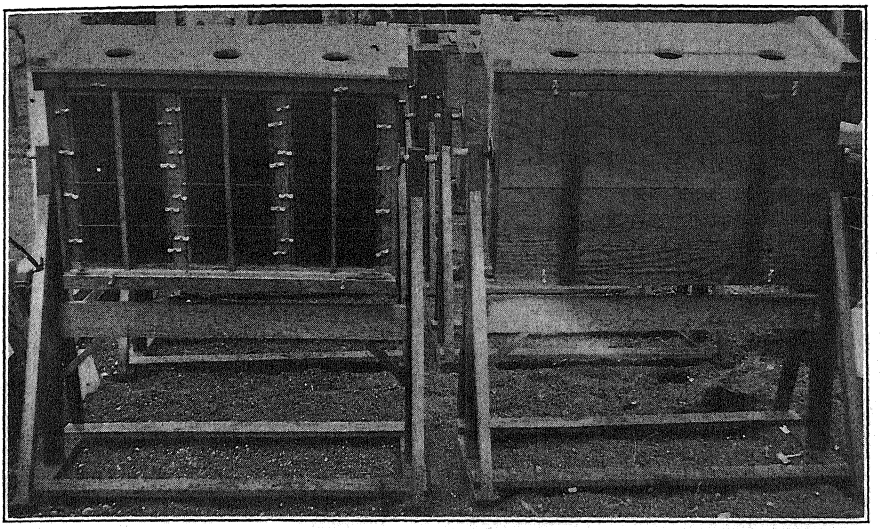


FIG. 2. Two root study boxes of the swinging type, one open and the other closed. In this model, measuring $1 \times 2 \times 3$ feet, plants can be grown for a longer time than in the smaller boxes. The arrow indicates the position of a hook by means of which the box can be fastened at any angle for microscopical observations.



FIG. 3. Batteries of small and large root study boxes as they appear in the greenhouses of the Experiment Station of the Association of Hawaiian Pineapple Canners.

box, and one or two pineapple crowns that had been previously dipped in some fungicidal solution were planted. The growth and development of the roots of these crowns may be followed as closely as necessary with a microscope of the Greenough type mounted on a Dermatoscope stand. The fungus, if present, appears as a whitish cottony mass filling the air spaces

between the root hairs. Its filamentous hyphae are quite long and touch at widely separated points, thus presenting a more or less tangled appearance among the root hairs. Intercalary or terminal swellings are often present. The former are turbinate cells (Sammelzellen), and the latter zoosporangia and hypnospores (Dauersporen) of the organism.

Pure, or rather approximately pure, cultures of the organism may be prepared as follows: Transfers of extremely small pieces of roots infected with this fungus may be made to roots of other pineapple plants grown in root-study boxes containing sterilized soil. Owing to the rapid growth of this fungus on young pineapple roots new transfers may be made within 48–72 hours before any of the other organisms are able to reach the regions of the root already occupied by *Rhizidiocystis*. A number of successive transfers made in this manner yield an approximately pure culture of the organism on roots and in the soil. Soil inoculated in this manner may be used for the inoculation of other sterile soil in studies of this type.

In order to study the morphology and behavior of the organism, clean specimens are of paramount importance. As it is difficult to wash adhering soil particles from the root without destroying the root hairs and the organism thereon, it is easier and preferable to grow clean roots. This may be done as follows: The root box may be filled either with sterile river sand free from colloidal particles or with soil, the former being preferable. After the plants have produced roots about 1 in. long the sides of the root box are opened and furrows are made with a sterile spatula or other such object starting from the tip of the root and terminating at the bottom of the box. Furrows should be made in the direction likely to be followed by the root and should measure about $\frac{1}{2}$ in. in thickness and depth. Extreme care should be taken in watering these boxes to avoid washing the surface soil downwards and filling up the furrows.

MORPHOLOGY OF THE ORGANISM

It has been quite difficult to identify this organism owing to its failure to grow on artificial media or on other natural media except living pineapple root hairs. Repeated attempts to grow it on the root hairs of pineapple plants grown in water cultures failed, notwithstanding the fact that the root hairs there were living and in good condition. The apparent cause for the failure of the organism to grow in the latter case is the excess water of the culture.

The results obtained from the root-box studies on the morphology of the fungus may be summarized as follows: (*1*) The organism produces abundant white mycelium packed in the air spaces between the root hairs of the plant (Fig. 4). The mycelium is evanescent, and almost immediately

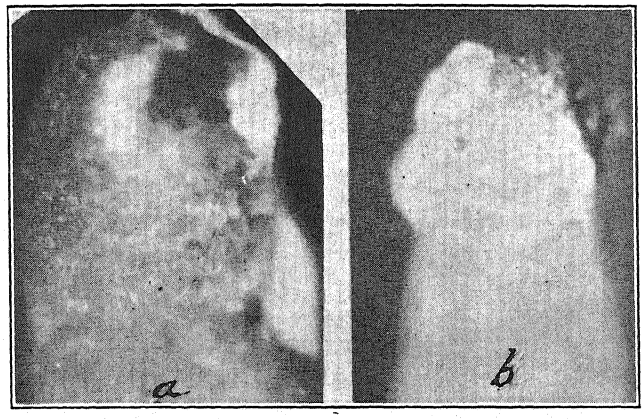


FIG. 4. Pineapple roots (×30) showing the white cottony mycelium of the fungus.

collapses and is no longer visible when the root upon which it is growing is cut off and removed from the soil atmosphere. (*2*) The hyphae are extremely fine and long, arachnoid in appearance (Fig. 6, *a* and *b*) with intercalary swellings (Sammelzellen) of spherical, triangular, oblong, or various other shapes and of various sizes (Fig. 6, *c*). The thin filamentous hyphae measure about 1 μ in diameter whereas the swellings may be from 3 to 6 μ. These swellings, technically known as turbinate cells, give rise to 1–8 branches, depending mainly upon their size. The protoplasm aggregates in the turbinate cells leaving the thin filaments empty. (*3*) The branches that are being produced from the turbinate cells produce at their extreme termini kidney-shaped zoosporangia (Fig. 5 and Fig. 6, *a* and *b*). These have never as yet been observed to give rise to zoospores. The zoosporangium when fully developed produces on its concave side an emission collar (Fig. 6, *a* and Fig. 7, *a, f, g, i, j, k* and *o*) from 1 to 2 μ in diameter and from 5 to 15 μ, or more, long. The zoosporangial emission collar serves the purpose of an appressorium to a certain extent besides that of discharging the protoplasmic contents that never develop into zoospores. The tube or emission collar enters the root hair of the pineapple plant (Fig. 8) wherein it discharges its protoplasmic contents which grow at the expense of the root hair, killing and assimilating the protoplasmic contents of the latter. (*4*) A mature zoosporangium gives rise to a hypnospore or resting spore, which is produced on the convex side of the zoosporangium (Fig. 9). The hypnospore starts as a tube, the end of which gradually enlarges until it becomes a sphere (Fig. 9, *a* to *h*). After it has reached full growth its surface becomes rough, the roughness increasing with age (Fig. 9, *i* to *o*). It happens occasionally that more than one hypnospore develops from the same zoosporangium; they may be produced either in

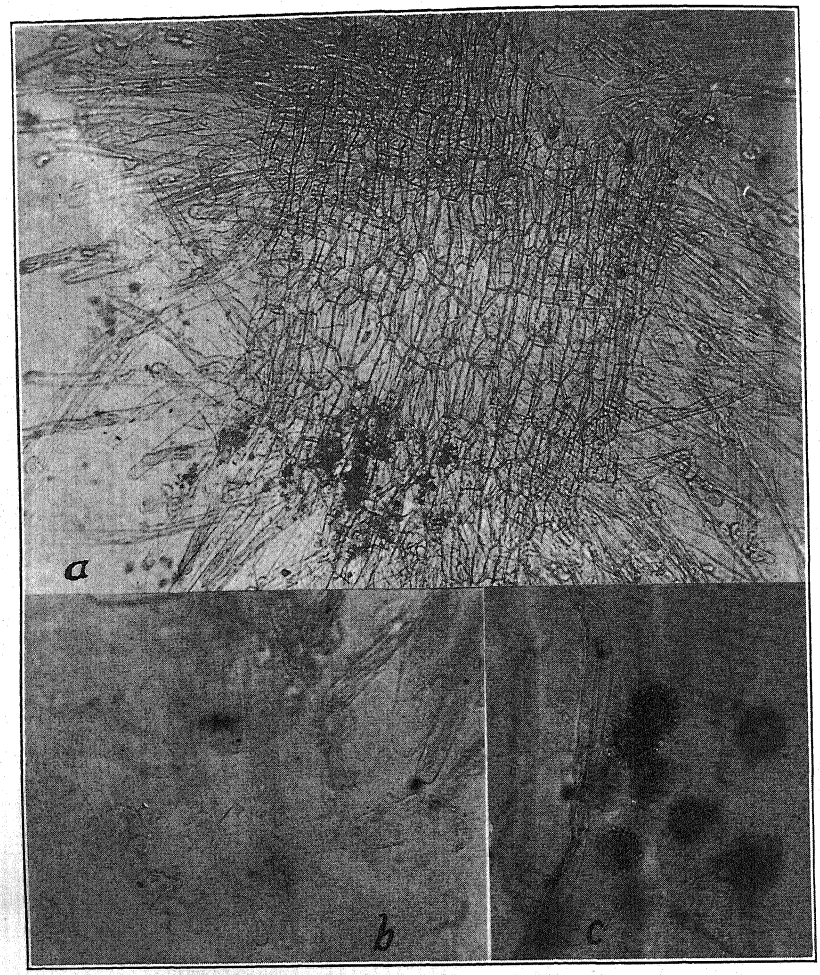


FIG. 5. (*a*) Portion of a pineapple root showing the zoosporangia of *Rhizidiocystis ananasi* attached to root hairs (×150). (*b and c*) Zoosporangia and hypnospores of the fungus attached to root hairs (×300).

chains (Fig. 7, *a* and *b*) or from two different tubes (Fig. 7, *c*, *d* and *h*). Not more than one of such hypnospores ever reaches maturity. Whether the hypnospore in this case represents the product of an asexual or sexual reproduction is not definitely known. The writer has observed quite often morphologically undifferentiated hyphae attached to such structures (Fig. 7, *j*, *k*, *l*, *m* and *n*) but, owing to the extreme minuteness of these hyphae, their relationship or union could not be definitely established. The details in the germination of hypnospores have not been fully investigated. It is

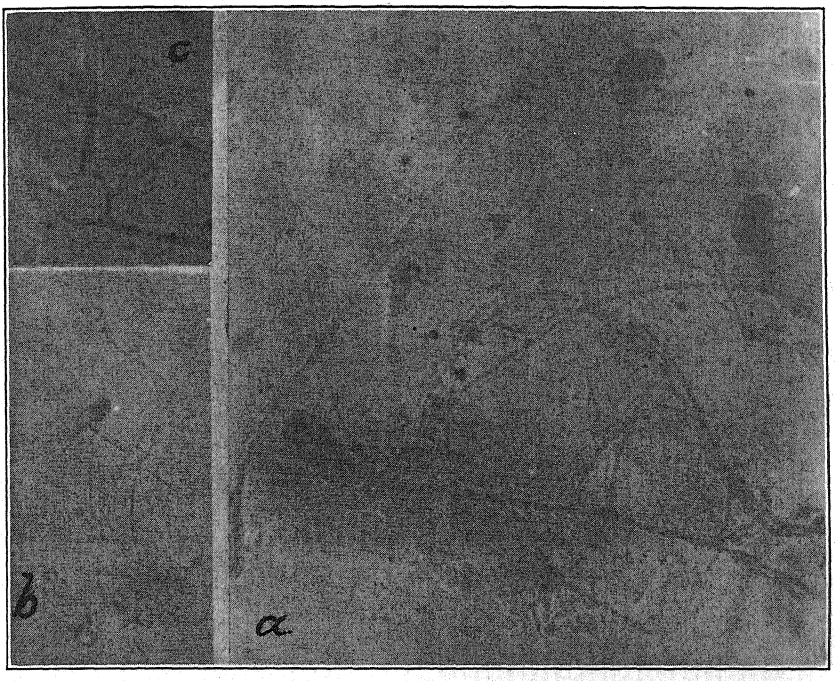


FIG. 6. (*a*) Mycelium, showing turbinate cells of different shapes and sizes and zoosporangia of *R. ananasi* (×600). (*b*) A young zoosporangium (×300). (*c*) A spherical turbinate cell with six branches (×1050).

certain that under the conditions of study the hypnospores did not produce zoospores, but in one or two cases the writer observed a flattened band or large hypha instead, broader at the base and pointed at the other end, which end extended but slightly beyond the rough shell of the hypnospore. This structure is filled with protoplasm and may be constricted at more or less regular intervals: turbinate cells (Sammelzellen) are formed at the constrictions. The hyphae that are produced on the turbinate cells and produce the zoosporangia are formed as soon as the turbinate cells are differentiated. As all these structures develop more or less simultaneously, the shell of the hypnospore becomes entangled in the newly produced mycelium, thus rendering extremely difficult the detailed examination of hypnospore germination. A germinating hypnospore seems to be between three and six times the size of one that is still attached to the zoosporangium. This is probably due to the fact that the shell of the hypnospore after cracking and opening occupies a greater area and therefore looks larger under the microscope. As hypnospores do not germinate at all seasons of the year, this fact alone increases the difficulties for their study. Germinating and germinated hypnospores have only been observed during the spring months, particularly

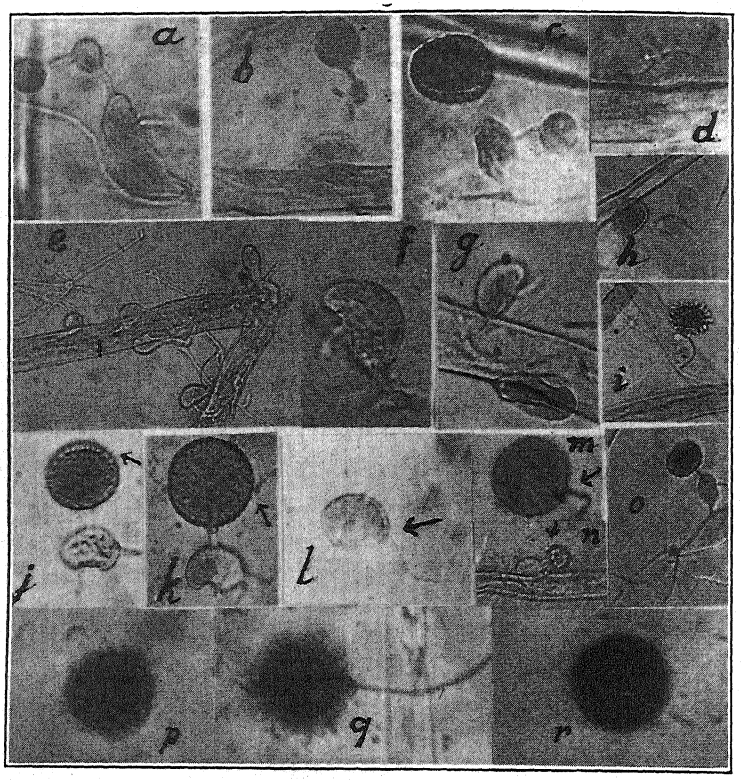


FIG. 7. (*a* and *b*) Zoosporangia with catenulate hypnospores (×600). (*c*, *d*, and *h*) Zoosporangia with two hypnospores (×600). (*e*) Root hair attacked by five zoosporangia (×300). (*f*) Zoosporangium (×1050). (*g*) Zoosporangium with emission collar turned upward (×600). (*i* and *o*) Zoosporangia with emission collar suspended in the air (×300). (*j*, *k*, *l*, *m*, *n*, *p*, and *q*) Hypnospores showing the point at which hyphae, morphologically nondifferentiated, may have fertilized them (*n*, ×300—others, ×600). (*r*) Hypnospore without its echinate shell (×600).

in April and May, and at temperatures ranging between 28° and 34° C. It is possible that they may germinate during the summer and autumn months but it is doubtful, as their germination has not yet been observed during such seasons.

CLASSIFICATION

The organism belongs, on the basis of its morphology, in the order Chytridiales and family Cladochytriaceae of the class Phycomycetes. As none of the known genera of this family manifests close similarity or identity in some of the striking features of this organism, the new genus

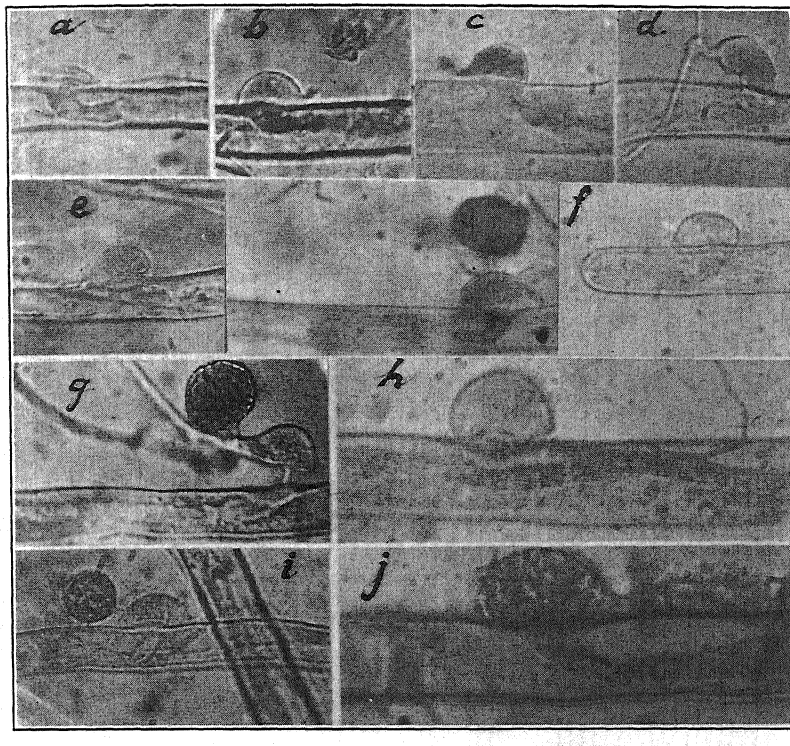


FIG. 8. (*a* and *b*) Thickened portion of the emission collar of the zoosporangium of *Rhizidiocystis* in a root hair (×600). (*c* to *j*) Zoosporangia attached to root hairs (*h* and *j*, ×1050; all others, ×600).

Rhizidiocystis[2] (from the Greek *rhizidion* = root hair and *cystis* = bladder) has been created.

[2] This organism has been recorded previously in Pineapple News (12) under the name *Nephromyces rhizidiophthoreus* Sid. The author believed at that time the generic name *Nephromyces* had never been used before in mycological nomenclature after having consulted Clements, "The Genera of Fungi"; Engler and Prantl, "Natürliche Pflanzenfamilien"; Wm. Englemann, 1897; Stevens, "The Fungi Which Cause Plant Disease"; and a few other works. After the name *Nephromyces* had been published the writer was able to secure a copy of Rhabenhorst's Kryptogamenflora; Fischer, "Die Pilze," IV Abt., Phycomycetes (5). There, on page 145 and under the title "Ungenau bekannte und sweifelhafte Gattungen der Hyphochytriaceae," the generic name *Nephromyces* Giard appears. The original paper (7) was immediately consulted and it was found that although the organism described by Giard as *Nephromyces* has some relationship to *Rhizidiocystis,* it represents, however, an entirely different organism from that studied by the present writer. Giard gave the name *Nephromyces,* meaning kidney fungus, to his organism because it was found in the kidney of certain ascidian species, whereas the present author assigned the same name on account of the kidney-shaped zoosporangia of the fungus.

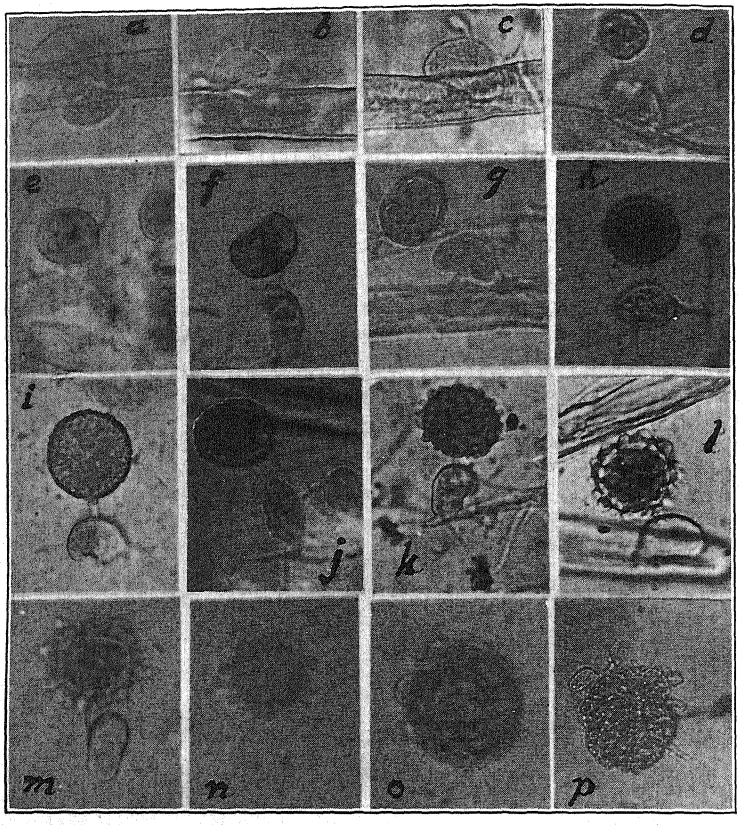


FIG. 9. (*a* to *m*) Different stages in the development of hypnospores (×600). (*o*) Mature hypnospore (×1050). (*p*) Germinating hypnospore not showing clearly on account of the roughness of the shell and mycelium surrounding it (×300).

Rhizidiocystis nov. gen.

Mycelium white and evanescent, composed of slender filamentous hyphae and turbinate cells of various shapes and sizes; filamentous hyphae 1 μ, or slightly more, in diameter; turbinate cells spherical, triangular, or irregular in shape, varying from 1 to 6 μ in diameter, and bearing from one to eight hyphal branches; kidney-shaped zoosporangia borne on the termini of the hyphal branches from the turbinate cells; zoospores are not produced, but the protoplasmic contents of the zoosporangium are discharged directly into the host cell through an emission tube, which is produced on the concave side of the zoosporangium as that organ matures; hypnospores are produced on the convex side of the zoosporangia, appearing as small tubes in the early stages, and later forming smooth spherical or ellipsoidal structures, which become rough and echinate as the hypnospores mature; hypnospores germinate to produce mycelium but never zoospores.

Rhizidiocystis ananasi nov. sp.

Mycelium white and evanescent, packed in the air spaces between the root hairs of the host plant; hyphae very long and slender, about 1 μ in diameter, extending from one root hair to another like spider webs; turbinate cells with one to eight hyphal branches; kidney-shaped zoosporangia, about 16 μ in length and 8 μ in diameter, produced at the ends of hyphal branches from the turbinate cells, and attached to the root hairs by means of an emission tube or suspended in the growing medium; emission tube, produced on concave side of zoosporangium, penetrates the root-hair wall and serves as means for the discharge of the contents of zoosporangium in a plasmodial state; the plasmodial mass grows within the root hair and kills and assimilates its protoplasm; hypnospores produced on concave side of zoosporangia, about 20 μ in diameter, smooth and tubular in the very early stages, spherical or slightly ellipsoidal and echinate in the later stages.

A strict parasite, which has been found only on living root hairs of pineapple grown in a natural soil habitat.

COMPARATIVE MORPHOLOGY OF RHIZIDIOCYSTIS AND OTHER MEMBERS OF CLADOCHYTRIACEAE

According to various taxonomists the outstanding characters that are more or less common to all members of the Cladochytriaceae are: (*1*) Very thin, filamentous, and evanescent mycelium; (*2*) turbinate cells (Sammelzellen) which vary in size and shape; (*3*) reproduction by means of either hypnospores or zoospores, or both; (*4*) sexual reproduction not definitely established, although it is possible that hypnospores may result from the union of male and female gametes.

Different investigators differ in their opinions of the phylogenetic position of this family. Bessey (1) considers it related to the Saprolegniaceae, its structural degeneration probably having resulted from hysterophytism and having affected the sexual reproductive organs more than the vegetative filaments. Gaümann (6) and Petersen (11), on the other hand, are of the opinion that Cladochytriaceae represent an ascending and not a descending group of organisms, their mycelium being better developed and more specialized than in the other families of Chytridiales.

There are quite appreciable morphological as well as physiological differences between the different organisms of this family, which complicate to a considerable degree their relationship and classification.

Cladochytrium (3, 10), which is the type specimen of the family, has very thin, filamentous hyphae from 1 to 2 μ in diameter and produces turbinate cells (Sammelzellen) which may become zoosporangia. The zoosporangia may produce zoospores which are liberated through a tube-like emis-

sion collar or may germinate directly with germ tubes instead of producing zoospores. *Amoebochytrium rhizidioides* Zopf (15), which is transitional between the Rhizideae and Cladochytriaceae, reproduces by aflagellate zoospores liberated from flask-like swellings with a tubular emission collar.

Nowakowskiella (2), *Physoderma* (14), and *Urophlyctis* (9) reproduce by zoospores or by hypnospores.

Rhizidiocystis behaves more like *Cladochytrium* than any of the other genera in the development and behavior of its zoosporangium; the zoosporangium germinates and its contents enter the tissues of the host directly without the formation of zoospores. The hypnospores (Dauersporen) of *Rhizidiocystis* are morphologically different from those of the other genera, being rough and to a certain extent echinate. The writer is of the opinion that the nonproduction of zoospores by the zoosporangia of *Rhizidiocystis* is due to change of habitat, namely, changing from an aquatic to a terrestrial mode of life, a condition forced upon it probably by its parasitic behavior.

PARASITIC BEHAVIOR OF RHIZIDIOCYSTIS

Rhizidiocystis ananasi is a parasite which is restricted to pineapple root hairs. It may be found quite often on the root tip where root hairs have not yet developed, but even in such cases it is superficial and has never been observed to invade the cells of this region. It is difficult to explain this high degree of specialization of the fungus. The root hairs are invaded by the emission collar of the zoosporangium, none of the other structures of the fungus entering the host tissues. It seems that these emission collars are highly chemotropic and it is possible that they locate root hairs in this manner. The angle formed by the emission collar with the zoosporangial wall is not always the same; angles varying between 45 and 90 degrees may be considered normal, but those that are as small as 15 and as large as 165 degrees are produced by movement of the emission collar in respect to a root hair which has either been invaded or is to be invaded. Emission collars have been observed to turn not only upwards and downwards in locating the root hairs of the host but also sidewise. All these tropisms of emission collars cannot be due to any single factor other than chemotaxis. Thigmotaxis may be excluded in this case on the ground that tissues other than root hairs are not invaded.

Whether the emission collar makes its way into the root hair by means of cytolytic enzymes or mechanical pressure has not yet been definitely established. After the root-hair wall has been penetrated, the emission collar enlarges in thickness at the point of entrance and immediately behind the root hair wall, forming a node, which tends to increase the holdfast of the organism on the root hair (Fig. 8, *a*, *b* and *i*). The writer has not been

able to ascertain the conditions that produce the enlargement of the fungal hypha at the point of entrance, that is, whether it is due to thickening in the wall of the collar or to an accumulation of protoplasmic contents. The zoosporangial contents then continue to grow inside the root hair by killing and gradually assimilating the protoplasmic contents of the host.

Rhizidiocystis ananasi makes a very prolific growth and is capable of attacking every root hair that has been produced or is in the process of development. Roots are literally denuded of their root hairs by this organism when grown in soils favoring its optimum development. It is true that there is no rot or destruction of the main root tissues. The root, however, grows slenderer as the destruction of its root hairs continues until it becomes atrophied and may persist in that condition until it dries up and dies, or is attacked by other fungi and destroyed. Roots that have become infected by *Rhizidiocystis ananasi* die very slowly; this is especially characteristic of plants growing in soils of low moisture content. With soils of high moisture content the damage is not so pronounced, as the roots grow more rapidly and consequently have more root hairs than the fungus can kill in a given period. It has been observed that with rapidly growing varieties of pineapples the damage to the root system caused by *Rhizidiocystis* is not so great as with slowly growing varieties (13). The reason is that the organism is not producing zoosporangia as fast as the roots are producing root hairs and as a result many of the root hairs are able to escape destruction. Old root hairs that may be dead have never been found invaded by *Rhizidiocystis ananasi*; it is invariably young root hairs that are invaded and killed. Inoculation studies with *Rhizidiocystis ananasi* have shown that practically all varieties and strains of pineapples are susceptible.

DISTRIBUTION

The organism is more or less restricted to pineapple fields. Repeated attempts were made to recover it from forest land, sugar cane fields, banana land, virgin land, and from cultivated land on which pineapples had never been grown; but none of the attempts were successful. In land that was planted to pineapples recently or within the last ten years, the presence of the fungus was detected in the majority of cases. Soil was obtained from a field that was planted to pineapples 20 years ago but never since then, and there the fungus was not found. The organism has been isolated from small as well as large fields, from fields that are situated in the center of the main pineapple districts, as well as from isolated fields. The latter are represented by such fields as the Kapalama substation, Mr. Meyer's field on the island of Molokai, Mr. Gay's field on the island of Lanai, and the Pearl City Fruit Company's fields in the Waianae district. Soil

samples were secured from areas inside and outside these fields; in the former case the organism was isolated, whereas results in the latter were negative. All the studies conducted to date indicate that the organism is intimately associated with pineapple plants; in all probability its other hosts, if any, must be exceedingly few or rare. Inoculation studies conducted on *Saccharum officinarum*, varieties Lahaina, Yellow Caledonia, D–1135, and H–109, kindly furnished by Mr. C. C. Barnum, of the Hawaiian Sugar Planters' Association Experiment Station, gave negative results. Other plants such as *Cajanus indicus*, variety New Era, Panicum (*Pennisetum barbinode*), and sisal (*Agave sisalana*) likewise gave negative results, indicating that the organism is not carried by any one of these plants that often precede the pineapple plant in the field.

That this organism occurs outside of the Hawaiian Islands is not improbable. If we assume that the pineapple plant is the only host of this organism, the latter must have been introduced into the Hawaiian Islands together with pineapple plants sometime in the past. If, on the other hand, the organism has more than one host, then again it is probable that the organism exists in other parts of the world where its hosts are distributed, unless such hosts are restricted to indigenous Hawaiian plants not growing elsewhere.

Its distribution to other parts of the world from the Hawaiian Islands is quite probable, as various countries have imported planting material grown in the Hawaiian Islands.

PROPHYLAXIS AND CONTROL

As it is difficult to combat the fungus once it has invaded a field, every effort should be made to prevent its introduction. There are a number of common-sense agricultural practices that are at the same time intimately connected with field sanitation. Before and after a field is planted the agricultural implements used for the purpose should be kept away from infested fields and as free as possible from accidental contamination. The planting material to be used should be taken from a field that has been kept free from infection and should be sterilized by some fungicide to safeguard against accidental contamination. The free movement of implements, animals and other carriers should be either prohibited or strictly supervised and all chances for dissemination of the infection be eliminated.

In cases of fields already infested with the fungus the best procedure for freeing them from the infestation is by means of dry fallow for some years, the extent depending upon the climate, physical properties of the soil, and other such conditions. Before an infested field is planted again to pineapples a test should be made for the presence or absence of *Rhizidiocystis ananasi* in the soil by means of root-study boxes.

Not knowing what other plants may serve as hosts of this fungus, it is unwise to recommend the eradication of certain plants and not of others. Volunteer pineapple plants, however, should under all conditions be destroyed.

SUMMARY

Rhizidiocystis ananasi nov. gen. et sp., a new member of the family Cladochytriaceae, order of Chytridiales, has been isolated from pineapple roots where it attacks and kills the root hairs. It is a strict parasite, not growing on any other living tissue of the pineapple plant or on any other plants that have been studied, nor on natural or synthetic bacteriological media.

It may, under favorable conditions, destroy all of the root hairs of a plant, produce atrophy of the main root tissues and cause a gradual drying up and death of the root or roots. There is no rot produced in the tissues of the root by *Rhizidiocystis ananasi* so long as such root or roots are kept free from secondary infection.

Rhizidiocystis reproduces by means of hypnospores. Whether these organs are gamic or agamic has not been definitely settled. The germination of hypnospores has not been studied in great detail owing to many complicating factors.

The zoosporangia of this organism have never been observed to produce zoospores. The emission collar of the zoosporangium penetrates the root hairs of the host, wherein it discharges its contents, which kill and gradually assimilate the protoplasmic contents of the root hair.

The hypnospore (Dauerspore) is produced on the convex side of the zoosporangium as a tube and later assumes the form of a smooth sphere. In later stages of its development, the sphere becomes rough and more or less spiny. Hypnospores do not germinate readily and under all conditions.

Turbinate cells (Sammelzellen), a common feature of all genera of the family Cladochytriaceae, are produced in abundance in various shapes and sizes. The hyphae are slender, arachnoid, filamentous, and evanescent.

The organism has not been reported outside of Hawaii, although is believed that it may occur wherever pineapples are grown.

The writer is indebted to Doctors A. L. Dean, J. F. Illingworth, G. H. Godfrey, and Mr. G. E. Paxton for their kind cooperation throughout these studies.

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THE DEVELOPMENT OF SOIL ROT OF TOMATOES DURING TRANSIT AND MARKETING

G. B. RAMSEY AND ALICE ALLEN BAILEY

The southern or winter-grown tomatoes, produced for marketing in the North during the winter and early spring months, frequently suffer great damage due to decay caused by the common soil fungus, Rhizoctonia. The disease is especially serious following heavy rains and is so obviously associated with wet soil conditions that the significant common name "soil rot" has been almost universally applied to describe this particular decay. The causal organism, *Rhizoctonia solani* Kühn (*Corticium vagum* B. and C.), appears to be present in all of the important trucking regions of the South. The amount of damage caused in any one season is entirely dependent upon weather conditions.

Rosenbaum[1] found soil rot causing considerable damage to Florida tomatoes during transit in 1918, and showed that the causal organism could spread through the wrapping from one fruit to another, thus causing decay of the neighboring stock, provided the fungus found entrance through a wound or through the stem scar. About this same time, Link and Gardner,[2] working on the market diseases of vegetables, reported that soil rot of tomatoes, "appearing as it does in shipments from almost all regions and especially the South and California, probably caused more losses in the market than any other single factor."

During the past few years, Weber and Ramsey[3] have found soil rot especially destructive in the low muck soil of Florida. They report that when wet and moderately cool weather prevails during February and March this disease often destroys a very high percentage of the fruit. Usually only those tomatoes touching the soil or hanging near enough to become spattered with water and soil during rains are affected. Only occasionally fruits hanging well up in the vine develop soil rot. For this reason staked tomatoes are much less subject to infection than those not staked.

Observations and studies made by the writers during the past eight years on the important receiving markets of the North show that in almost every shipping season the tomatoes from Florida, Texas, Mississippi, California,

[1] ROSENBAUM, J. The origin and spread of tomato fruit rots in transit. Phytopath. 8: 572–580. 1918.

[2] LINK, GEO. K. K., and MAX GARDNER. Market pathology and market diseases of vegetables. Phytopath. 9: 497–520. 1919.

[3] WEBER, G. F., and G. B. RAMSEY. Tomato diseases in Florida. Fla. Agr. Exp. Sta. Bul. 185: 61–138. 1926.

and Mexico are subject to more or less serious loss from soil rot. Inspection certificates issued by the Food Products Inspection Service of the United States Department of Agriculture show that cars of tomatoes having from 5 to 15 per cent of decay due to soil rot are not uncommon during wet seasons, and occasionally the decay may range as high as 60 per cent. A random selection was made of certificates covering 26 cars of Florida tomatoes that showed soil rot when inspected in the northern markets during the spring of 1926. In these cars there was an average loss of over 7 per cent due to this disease. In the best car there was an average of 2 per cent decay, while in the poorest the range was 15 to 50 per cent in the individual crates, the average for the car being 35 per cent.

These data show that, under favorable conditions, soil rot is one of the most destructive tomato diseases affecting southern stock. In view of its importance it seemed desirable to make a study of this disease as it occurs during transit and in the market, as well as in the fields and packing houses. The results herein reported cover field and packing-house investigations in Florida, Texas, and Mississippi; test shipments from these States; and laboratory and market studies made at Chicago from 1926 to 1928.

The early infections in the field are characterized by small circular brown spots on the lower half of the fruit. Usually the spots which are one-fourth inch in diameter and larger show narrow alternating zones of light and dark brown tissue which form definite concentric rings (Fig. 1, A). This is characteristic of spots up to one inch or more in diameter on green fruit in the field but, during transit, as the tomatoes ripen and the fungus makes more rapid growth, these concentric rings are obliterated (Fig. 1, B). On the market, soil rot appears on the fruit as large, brownish or reddish brown lesions (Fig. 1, C). In some instances, however, the lesion has a muddy-brown cast due to the retention of part of the green color in the decayed area as the fruit ripens. Cream-colored or light-brown mycelium is often visible on the surface of the tomatoes when they arrive on the market, and may penetrate the moist wrappings and form irregular brown sclerotia on them as well as on the fruit. Frequently the paper wrapping becomes firmly attached to the tomato at the place where such mycelial mats occur (Fig. 1, D). The decay produced is firm at first, but becomes softer and more watersoaked in appearance as the fruit ripens and the fungus penetrates deeper into the tissues. In the larger lesions the epidermis ruptures and the crack becomes filled with brown mycelium and sclerotia.

The early symptoms become visible on the fruit in the field about the third day following a rain. So long as the top soil is fairly dry Rhizoctonia does not come to the surface or cause appreciable trouble, even though many of the fruits may be in contact with the soil. Where the plants are

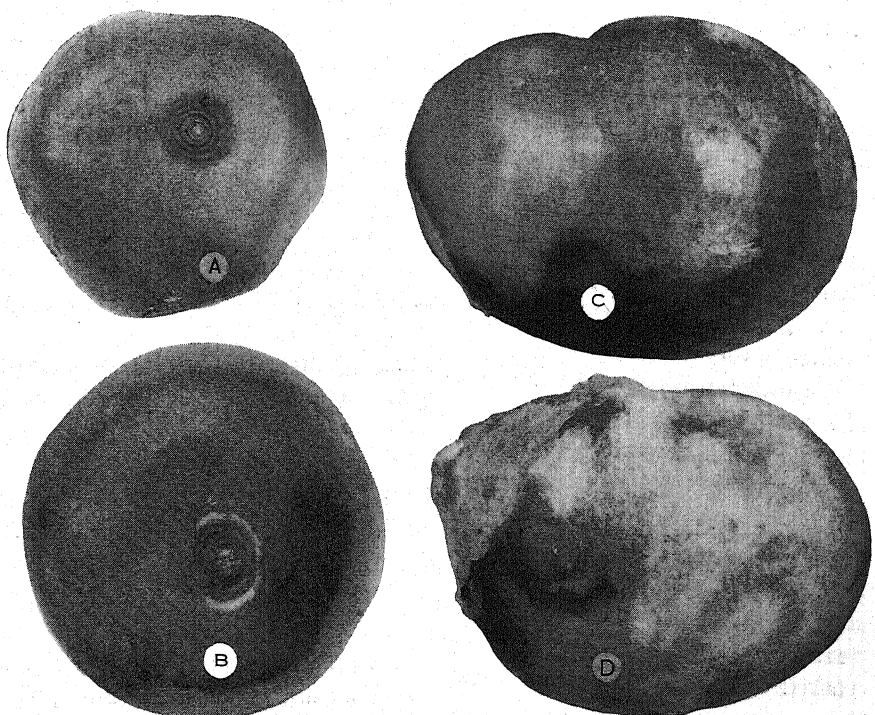


FIG. 1. A, Early stage of soil rot of tomatoes showing definite concentric rings. B, A later stage showing development of lesion and obliteration of rings. C, Soil rot as it appears in the receiving market. D, Advanced stage showing surface mycelium and part of moist wrapping attached to the fruit.

large and spreading and the foliage is dense enough to hold the soil moisture and maintain a high degree of humidity underneath the vines, soil rot may occasionally be found for long periods following rain. This is especially true in localities where heavy dews which drench the plants are common. Under ordinary conditions, however, most of the affected tomatoes decay, and within a week or ten days following the rainy period have disappeared.

Most of the important commercial growers of tomatoes recognize the danger from soil rot and take precautions to see that little affected stock is sent to the packing house. Following heavy rains during the packing season, the laborers are instructed to discard the decaying tomatoes in the field. Those showing large lesions are eliminated, but in many instances a rather high percentage of affected tomatoes reaches the packing house. For example, in April, 1926, at Goulds, Fla., a careful inspection of one grower's stock showed 30 per cent soil-rot infection of fruit in the field boxes

brought to the packing house. After this stock was sorted and graded ready for packing, a reinspection showed that there was still 18 per cent of the graded fruits affected by this disease. In other words, approximately 12 per cent of the stock reaching the packing house showed lesions large enough to be plainly visible and was discarded in the commercial sorting and grading, whereas the remaining lesions were so small that they were not seen, or at least not considered likely to cause decay during transit and marketing. It is these small lesions, however, which cause so much loss during marketing. In some instances it has been found necessary to stop packing tomatoes for a day or two in order to allow the decay to develop to a stage which can be easily recognized and sorted out before packing.

In order to determine the rate of development in transit of these small lesions which were overlooked by the graders, as well as some larger ones, test shipments were made to Chicago.

EXPERIMENTAL SHIPMENTS

In selecting tomatoes for experimental shipments, precautions were taken to exclude all fruit that showed mechanical injury or diseases that might influence the rate of development of soil rot during transit. All tomatoes were mature, green commercial stock and were wrapped and packed in six-basket crates or lugs, according to the commercial practice followed at the point of origin of the various shipments. Because of the rapid breakdown of tomatoes which had lesions 20 mm. or more in diameter when packed, only those with lesions covering a range in size below 20 mm. were selected for shipping. The diameters of all spots were measured in millimeters and the sizes marked on the tomato with India ink. On arrival at Chicago the tomatoes were unpacked and the spots remeasured in order to determine the amount of development during transit. In this manner, accurate data were obtained on the rate of development of lesions of various sizes. The percentage of new lesions developed in transit was noted, and their average diameter also obtained. For the purpose of determining the amount of spread in transit, healthy tomatoes and infected fruits were mixed in the experimental shipments.

For detailed study, special consideration is given to 522 tomatoes that were included in experimental shipments. Of these, 168 showed soil-rot lesions when packed at the shipping point, while the remaining 354 healthy fruits were interspersed among the diseased stock. Seven of these infected fruits and 44 of the healthy ones, or 9.8 per cent of those shipped, developed new soil-rot lesions during transit. There was a total of 337 soil-rot lesions present on the 212 affected tomatoes when they arrived at Chicago, 82 of which were new lesions. On arrival, 9.7 per cent of the new lesions

and 12.7 per cent of those present on shipping, were contaminated, most of them with bacteria or Rhizopus, and one with Melanconium.

All but 12 of the new lesions (14.6 per cent) developed in transit as a result of contact with the wet papers of infected fruit. Approximately one-half of the new lesions occurred where there was no evident break in the skin. In 16 per cent of the infections a wound or bruise was the place of entry, in 25 per cent of the cases the organism entered through the stem scar, and in 7.4 per cent through the blossom scar. Besides the 70 tomatoes which showed new lesions due to spread in transit 55 were covered with mycelium on arrival but showed no lesions, and the wrappings of 22 more were moist and overgrown with mycelium and sclerotia. These 77 fruits were replaced in their wrappers and held for a while at room temperature. In four days, 6 of them developed Rhizoctonia lesions under the mycelium on the surface of the fruit, and 2 developed lesions which were contaminated with bacteria. Three additional pure soil-rot lesions developed in 13 days. The new lesions developed on both green and ripe fruit in transit and storage. These data indicate that Rhizoctonia can enter the tomato fruit through an apparently sound epidermis, if in contact with it long enough. Field observations lead to the same conclusion.

In table 1, the increase in diameters of soil-rot lesions of various sizes during transit and the average daily increases in diameter are shown. For

TABLE 1.—*The increase in diameters of soil-rot lesions on tomato fruits during transit*

Diam. of lesion at shipping point (mm.)	No. days in transit	Diam. of lesion at end of transit period (mm.)	Increase in diam. of lesion (mm.)	Average daily increase in diam. of lesion (mm.)
0	5	11.6	11.6	2.3
	7	24.7	24.7	3.5
1–5	5	17.2	13.2	2.6
	7	29.9	25.8	3.7
6–10	5	30.8	22.5	4.5
	7	43.3	35.6	5.1
11–15	5	42.0	29.8	6.0
	7	55.2	42.6	6.1
16–20	5	47.0	29.0	5.8
	7	60.0	42.3	6.0

convenience, the spots were considered in five groups, the 0 representing the new spots that developed during the transit period. From this table, it will be seen that the rate of development was much more rapid in the larger

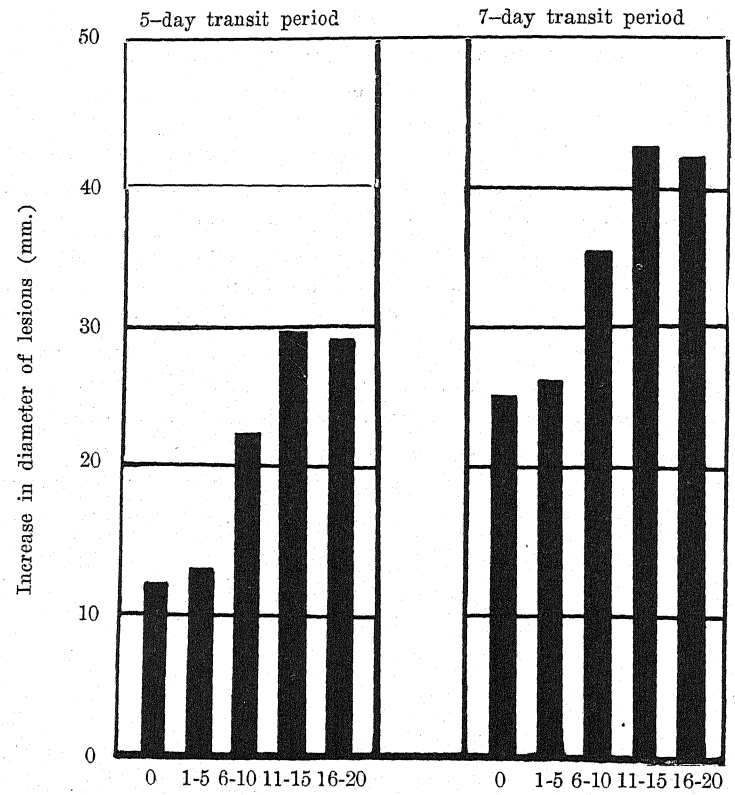


FIG. 2. Average total increase in size of soil-rot lesions on tomato fruits during transit.

spots than in the smaller. The maximum rate was attained in spots approximately 15 mm. in diameter. Spots of this size enlarged about twice as fast as those 5 mm. or less in diameter when packed. The slowest of these, however, developed rapidly enough to destroy the marketability of all affected stock, as shown in figure 2, which shows graphically the average total increase in size for 5- and 7-day transit periods. A transit period of 5 days would be considered reasonable for most shipping points in the States under consideration, yet in this moderate time the new spots enlarged to 11.6 mm. in diameter (Table 1). The visible spots up to 5 mm. in diameter, which constitute the majority of those overlooked in commercial grading and packing, attained an average diameter of 17.2 mm. by the time they reached the market after 5 days in transit. However, the fact that spots 30–40 mm. in diameter are found on tomatoes on arrival at the market indicates that spots larger than 5 mm. are sometimes included in commercial shipments.

INOCULATION EXPERIMENT

The following data obtained from artificially infected fruit give further evidence of the rapid development of soil rot in transit. In all test shipments of naturally infected tomatoes, the lesions were for the most part 5 mm. or larger in diameter. In order to determine the rate of development when infection through wounds takes place just previous to packing, one lug of inoculated fruits was sent to Chicago from Texas.

Seventy-three mature green tomatoes, selected for freedom from disease, were inoculated by inserting a small bit of mycelium into a slight scalpel wound made on the side of each fruit. These tomatoes were kept at a temperature of 75°–80° F. for 15 hours before they were wrapped and packed. At the end of this time, slight discoloration and decay were visible along the edges of the inoculation wounds. On arrival at Chicago after five days in transit, an examination showed all of the tomatoes to be seriously affected with soil rot. The lesions found varied from 7 to 43 mm. in diameter, and from 5 to 20 mm. in depth. The average diameter of all spots was 20.4 mm. After the spots were remeasured, the tomatoes were again wrapped and packed in the lug and kept for two days at a temperature of 66°–72° F. On examination this time, the average diameter of all spots was 38.1 mm., and the decay ranged from 20 to 40 mm. deep.

SUMMARY

1. Soil rot is caused by the common soil fungus, *Rhizoctonia solani* Kühn, which appears to be present in all of the southern trucking regions.

2. This disease is sometimes responsible for great losses in tomato shipments during transit and marketing, as well as in the field and packing houses.

3. The amount of damage is directly related to weather conditions. Heavy rains or successive moderate rains that keep the surface soil wet favor the development of soil rot, whereas, under moderately dry conditions where the surface soil is dry, the disease is of no economic importance.

4. Experimental shipments of southern tomatoes made during 1926–27 and 1927–28, averaged 9.8 per cent new lesions on arrival at Chicago. About 50 per cent of these lesions developed in both green and ripe fruits which showed no break in the epidermis. In the rest of the cases, the organism entered through wounds and stem and blossom scars. The new lesions averaged 11.6 mm. in diameter after five days in transit.

5. The rate of development of soil-rot lesions varied directly with the size of the spots, the maximum rate occurring in those approximately 15 mm. in diameter when the shipment started.

6. The visible spots up to 5 mm. in diameter, which constitute the majority of those overlooked in commercial grading and packing, attained an average diameter of 17.2 mm. in five days transit.

7. A shipment of inoculated tomatoes showed 100 per cent infection. The lesions produced averaged 20.4 mm. in diameter and ranged from 5 to 20 mm. deep at the end of five days in transit.

OFFICE OF VEGETABLE AND FORAGE DISEASES,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

THE OCCURRENCE OF SCLEROSPORA GRAMINICOLA ON MAIZE IN WISCONSIN

WM. H. WESTON, JR.[1]

In November, 1925, Melhus and Van Haltern (3), at the Iowa Agricultural Experiment Station, reported that they had successfully inoculated seedlings of corn artificially with *Sclerospora graminicola* (Sacc.) Schroet. from the green foxtail grass, *Setaria viridis* (L.) Beauv. After describing the effect of the disease on the inoculated maize plants and noting that the production of conidiophores and conidia occurs in the case of corn only in the seedling stage and is much less even at its maximum than the ordinary sporulation on *Setaria viridis,* they state: "This probably explains why no one has recorded the presence of this disease [*i.e.*, on corn] before."

In view of the interest which any such record necessarily would deserve it seemed desirable, even after a lapse of some years, to report an earlier case of the occurrence of this disease on corn. Accordingly, in the summer of 1928, the writer prepared a manuscript embodying these records and also the conclusions arrived at from his own study of the diseased material from Wisconsin. Before this manuscript could be put into type, however, there appeared the detailed publication on this subject by Melhus and his associates (5) following their previous brief abstract (4). These publications make an extended discussion of the matter unnecessary, but it is believed that the historical value of the following paragraphs still warrants their publication.

On July 8, 1921, R. B. Streets, while studying at the Field Laboratory of the Department of Plant Pathology, of the University of Wisconsin, near Sturgeon Bay, Door County, Wis., collected material of the conidial stage of a downy mildew on leaves of young Golden Bantam sweet corn on the farm of S. T. Learned. As the conidiophores and conidia when examined under the microscope were obviously those of the genus Sclerospora, two possibilities suggested themselves; first, that the fungus might be one of the four or five Oriental conidial species of Sclerospora so severely destructive to maize, which, in some way, had been introduced into the United States and was starting to develop and spread on corn; or second, that the fungus might be the widely distributed *Sclerospora graminicola* common in that

[1] Formerly Pathologist in Charge of Downy Mildew Investigations, Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, who expresses his thanks to Dr. C. R. Ball and Dr. H. B. Humphrey, of that Office, for kindly placing at his disposal progress reports, correspondence, and other material relating to the subject of this note.

region on *Setaria viridis* and from this grass, transmitted by chance, under conditions unusually favorable for infection, to occasional maize plants near by.

A preliminary study of this material by Dr. Otto Reinking and G. A. Ocfemia, who had known the destructive Philippine downy mildew of maize in the Philippine Islands, led them to believe that, in all probability, the fungus was not that species of Sclerospora. As the writer, then Pathologist in Charge of Downy Mildew Investigations in the Office of Cereal Crops and Diseases, of the Bureau of Plant Industry, had recently returned from two years of study of the Sclerosporas of maize and related crops in the Philippines, Dr. E. M. Gilbert sent to him, also, some material which he, after examination and comparison, agreed was certainly not the destructive Oriental species. This material will be more fully discussed later.

FIELD SURVEYS AND EXPERIMENTS IN WISCONSIN

The infection noted by Mr. Streets did not spread to other plants of maize but gradually disappeared after he had collected nearly all of the leaves of the plants examined which showed the sporulation of the fungus. Further study of the situation seemed highly desirable, however, both to establish the identity of the fungus and to determine whether it would spread and maintain itself on maize. Accordingly, Mr. Streets, in the summer of 1922, was engaged to study further the situation in Wisconsin and at the close of his work he made a report to the Office of Cereal Crops and Diseases, the main results of which may be outlined as follows:

Beginning about the middle of August he carried on a field survey in the vicinity of Madison, Wis., in order to see if the same transmission of the causal organism might be taking place from the Setaria so commonly infected by *Sclerospora graminicola* thereabouts, but found none of the fungus on either host at that time. Proceeding to Sturgeon Bay on August 24, Mr. Streets stayed until the middle of September, making a very careful field survey and also attempting natural inoculations under field conditions. At that time, sweet corn again had been planted in part of the same plot where he had found Sclerospora on corn the preceding year and in this field, as Mr. Streets reported, "the plot was examined plant by plant on two different occasions but no trace of Sclerospora was found on the corn. Adjacent fields within a radius of one-half mile were all scouted two rows at a time by examining every stunted or abnormal plant; more extensive scouting was done also in different parts of the peninsula." "In brief, my best efforts failed to find the fungus on corn at this season (1922), when, according to prevailing weather conditions, it should have been fruiting abundantly if it had survived the dry part of the summer in an active condition."

In addition to this extensive survey, Mr. Streets made attempts to secure infection by natural inoculation in the field from *Setaria viridis* to maize. On August 25, for example, as there was too little conidial material on Setaria for experiment, the oospore-containing tissue of host plants was "spread on the ground along the rows of corn (recently planted Golden Bantam) which had not yet emerged." The young corn plants grew up through this material but no visible infection had occurred by September 11, when Mr. Streets made his last inspection, nor by September 30 when examined by others.

Mr. Streets made a second survey of the district in the summer of 1923, working somewhat earlier in the season when the conditions were better for the transmission of the fungus to corn than they had been in the latter part of the previous summer. In July, he intensively surveyed many fields around Madison, Wis., especially those of sweet corn, but found no sign of infection by the downy mildew, anywhere, even on such especially favorable fields as some of the college experimental plots which were planted to varieties of sweet corn and had numerous plants of Setaria infected by the downy mildew near by. The weather was hot and dry, however, and even on the Setaria there was very little spore formation by the fungus.

Proceeding to Sturgeon Bay in the latter part of July, 1923, Mr. Streets continued an intensive survey in the same locality where he had found the infection on maize in 1921. The situation was better than in the previous summer of 1922, as the weather was more favorable and the Sclerospora was as abundant as ever on the Setaria. Yet even in the field where the original infection had been found, and where Golden Bantam was again planted in three sections of different ages, the youngest being then about six inches high, no infection by the downy mildew was found. The survey was extended into August and through a considerable part of the surrounding territory so that no portion of the county was overlooked, but no cases of infection were found on maize.

Mr. Streets also made attempts at this time to secure natural inoculation in the field. In July, 1923, for example, he planted heavily infected plants of Setaria very close to young Golden Bantam corn so that the leaves intermingled with those of the corn plants of different ages. The weather was not particularly favorable for natural infection, however, so no infection resulted, and some of the transplanted Setaria died. In August, this attempt was repeated under much more favorable weather conditions when there were several periods of considerable rainfall and high humidity favorable to infection and when abundant new lesions were appearing on Setaria itself. Yet no infection of maize was secured in this manner. Early in August, also, attempts were made to infect young maize plants artificially

by placing conidia from Setaria in drops of moisture on the leaves and by spraying the conidia on to the maize from an atomizer, subsequently protecting the inoculated plants by covering them with inverted fruit jars and shading them from direct sunlight. Not one of these attempts was successful.

The work just reported, even though largely negative, did bring out the following points. The species of Sclerospora found on maize at Sturgeon Bay in the summer of 1921 did not spread further on that host during that summer nor did it appear again on that host in that locality in the summers of 1922 and 1923. Moreover, no other cases of infection by this downy mildew on this host were encountered in that whole county nor around Madison during those two summers.

STUDY OF INFECTED MAIZE FROM WISCONSIN

Mention was made previously of the specimens of Golden Bantam sweet corn sent from Wisconsin to the writer by Dr. E. M. Gilbert. The material consisted of a few small pieces of dried blades of leaves, somewhat discolored but showing occasional narrow linear elongate streaks, probably once etiolated but now somewhat brownish, on which here and there on the lower leaf surface a close, rather granular, grayish to buff colored down could be seen. In general these linear streaks were not so broad nor so extensive as those which characterize systemic infection of maize by the conidial Sclerosporas of the Orient; rather did they resemble those typical of the local infection spotting which the writer and G. F. Weber (11) found on the leaves of *Setaria* (*Chaetochloa*) *magna* in Florida. The down from the leaves, when carefully mounted, was found to consist of Sclerospora conidia and conidiophores in rather poor condition for comparative study; first, because they were obviously the last scanty remnants of the previous night's sporulation dried by the morning sun and collected during the day, and second, because they had become overrun with an Aspergillus by which the leaf specimens, probably through inadequate drying, had become moulded here and there.

On microscopic examination even this material showed clearly that the fungus was not one of the destructive conidial Sclerosporas attacking maize in the Orient. All of these species have relatively large conidiophores, with an extensive and elaborate branch system, and a definite basal cell or foot, while in this Wisconsin material the conidiophores were short and stocky with small, irregular branches and no clearly defined basal cell. The conidia also were quite distinct, for they were small in size and rather broadly ellipsoidal in shape, even smaller than those of the smallest Oriental species, *S. javanica* Palm (7) with its rather rotund conidia, the bulk of

which are 21 to 27 μ in length by 15 to 20 μ in diameter, and strikingly different from the much larger and more elongate conidia of the other Oriental species,—*S. maydis* (Rac.) Butl. (1), with spores mostly 28 to 45 μ long by 16 to 22 μ diameter; *S. sacchari* T. Miyake (2, 6), with spores mostly 35 to 43 μ long by 16 to 22 μ diameter; *S. philippinensis* Weston (8), with spores mostly 31 to 37 μ long by 15 to 20 μ diameter; and *S. spontanea* Weston (9), with spores mostly 39 to 45 μ long by 13 to 18 μ diameter. Even more distinctive in mature conidia of the Wisconsin material was the presence of a modified portion of the wall at the apex, the so-called papilla of dehiscence through which the zoospores would have emerged from these conidia (*i.e.*, zoosporangia) on germination, for the conidia of all the Oriental species just enumerated have true conidia which utterly lack any papilla of dehiscence and always germinate by means of germ tubes.

All of these characteristics of the Wisconsin material, namely, the short stocky conidiophores with their small irregular branch system and lack of any distinct basal cell, and the small broadly ellipsoidal conidia (zoosporangia) with the apical papilla of dehiscence, were very strong indications that the fungus was *Sclerospora graminicola* (Sacc.) Schroet. On May 22, 1922, after having examined the material, the writer wrote Dr. Gilbert that "The Sclerospora is not the typical Philippine species nor is it like the other forms in the Oriental tropics that have been described from Java, India, and Formosa. It most closely resembles *Sclerospora graminicola* which is the common parasite of Setaria (Chaetochloa) collected by J. J. Davis in your region." However, the caution attending an identification of poor material prompted adding "there are, however, points of difference which may mark it as distinct from that species but it is hard to be sure from dried material collected during the day time."

These differences were a seemingly greater irregularity of the branch system and apparently smaller size of the conidia, but later after going over these points in careful comparisons with material of *S. graminicola* on Setaria from Minnesota, North Dakota, and other localities in the United States, the writer became entirely convinced that these points of difference were not significant and that the fungus on maize in Door County, Wis., was in fact this species. Indeed, the size of the conidia in the Wisconsin material, in which the great proportion of the spores (for example, 72 out of 100) fell in length classes between 15 and 20.9 μ and in diameter classes between 11 and 14.9 μ (94 out of 100), was found to be quite in agreement with similar measurements of the species on Setaria in Minnesota gathered fresh during nocturnal sporulation but killed in Flemming's weaker solution and mounted in dilute glycerine, or with those of this species on Setaria in North Dakota mounted in water and then dilute glycerine after previous

moistening with alcohol, from herbarium specimens, that had been collected during the day and merely dried. Probably, as the writer later pointed out (10, p. 777), if measurements were made of conidia when shed at night in dew or other moisture under optimum conditions the modes of length and diameter would be increased by one 2 μ class of size, but as all of the material compared was fairly comparable in preparation the close agreement in size seems dependably significant.

Mr. Streets' discovery of *Sclerospora graminicola* on Golden Bantam sweet corn in Wisconsin in 1921 is of especial interest as the first recorded, though unpublished, account of the infection of this host by this parasite in nature. As the evidence presented by this one case of natural infection is in agreement with the evidence of the extensive artificial infections secured by Melhus and Van Haltern, it seems thoroughly justifiable to expect that from time to time cases of transmission of this species of Sclerospora to maize from near-by gramineous weeds will be encountered in nature elsewhere.

It is worthy of note in connection with the occurrence of *Sclerospora graminicola* on sweet corn in Wisconsin that in Iowa Melhus *et al.* (5, p. 331) observed *Sclerospora graminicola* occurring naturally on corn in the field only twice, once on dent corn seedlings at Boone in 1925, and once on Golden Bantam sweet corn nearing maturity at Story City in 1927. It also is interesting to note their statement that "A survey made in June, 1927, which included readings on 3,350 corn plants (dent, pop and sweet) located at 19 places in 16 of the north central counties of Iowa, revealed no positive symptoms of infection of Sclerospora."

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A SCALE FOR MEASURING AREAS OF RIBES LEAVES[1]

ROBERT S. CARUTHERS

In studying plants, occasions arise when it is necessary to determine the surface areas of their leaves. The planimeter method of measuring the areas of an extensive series of leaves is not only tedious, but also entirely unsuited to periodic measurements of growing leaves. When an accuracy of 95 per cent satisfies the purpose for which the measurements are undertaken, the planimeter method may be replaced by the use of "leaf-area scales" (Fig. 1), which can be applied without injury to growing leaves,

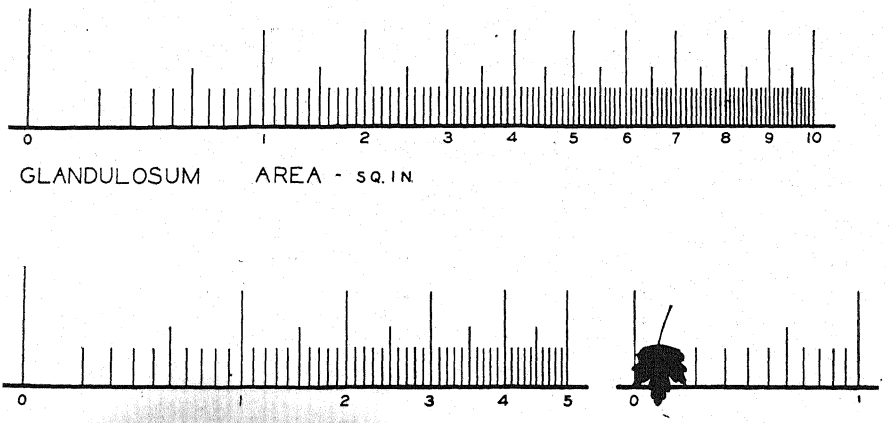


FIG. 1. Leaf area scales for *Ribes glandulosum* and *R. rotundifolium* constructed from logarithmic graphs of average areas in corresponding width classes, figures 4 and 5.

and by means of which areas can be read directly from one or two lineal dimensions. The construction of these scales is a simple matter. The scales illustrated in figure 1 were developed for determining the areas of leaves of currants and gooseberries (Ribes spp.) in connection with the experimental eradication of these plants to protect white pine from blister rust (*Cronartium ribicola*). The leaves measured for constructing the scales were collected from plants growing under average conditions of shade and moisture in different localities near North Hudson, New York.

As the leaves of a given species are usually somewhat similar in shape, general relations exist between their areas and lineal dimensions. In basing a leaf-area scale on one lineal dimension, greatest accuracy will result from the use of that dimension offering the greatest coefficient of variability. If

[1] The writer is indebted to Mr. G. B. Posey for direction and assistance in the preparation of this paper.

1 to 3 inches represents the range of lengths for normal leaves of a certain species and 1 to 4 inches the range of widths, obviously the width will have the greater influence on area and will furnish the longer, and therefore the more accurately readable scale.

A relation existing between the area and a transverse dimension of a leaf must make itself apparent in the measurements of the area and the given dimension for a representative sample of the leaves of the species. If a series of width measurements are plotted on regular cross-section paper against corresponding areas, and the points lie on a smooth curve, a relation exists between the area and width which is shown graphically by the shape of the curve and analytically by the equation of the curve. It may be said, how-

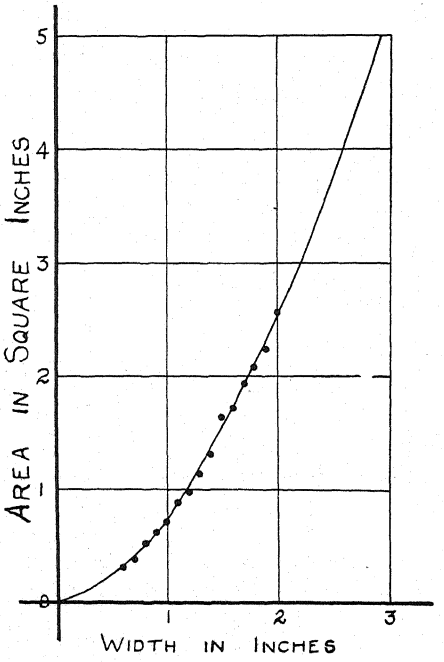


FIG. 2. Graph showing the relation between the areas and widths of leaves of *R. rotundifolium* plotted from the average area in each corresponding width class (Table 1).

ever, that abnormally shaped leaves are often of too frequent occurrence to allow a small sample of leaves to establish the average relation between a lineal dimension and the area. Even though a sufficiently large sample of leaves were measured to include all possible variations in leaf shape, weighted as to frequency of their occurrence, still it would be difficult to pass a curve through the plotted points which would represent the average relation. In most cases the points will be found to deviate too greatly from the curve to permit its accurate location without the use of detailed mathe-

matics. However, if an average area is determined from the measurements of 10 leaves for each tenth-of-an-inch width size class, the data will be condensed to represent more nearly average conditions. If these values are plotted on regular cross-section paper, a curve may be drawn by eye through the points. Such curves have been determined for two species, *Ribes rotundifolium* Michx. and *Ribes glandulosum* Grauer (Figs. 2 and 3). Average

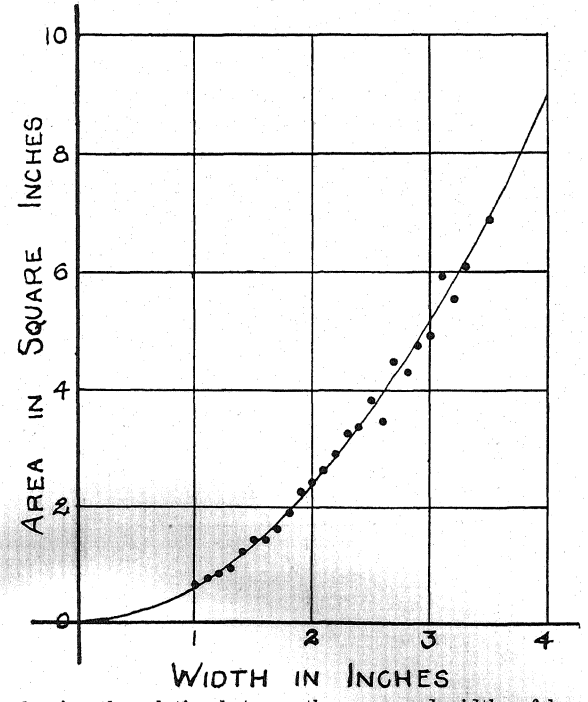


FIG. 3. Graph showing the relation between the areas and widths of leaves of *R. glandulosum* plotted from average area in corresponding width class (Table 1).

areas were determined for each tenth-of-an-inch width size class from 0.6 to 2.0 in. for *R. rotundifolium* and from 1.0 to 3.5 in. for *R. glandulosum* except for the 3.4-in. size, which was lacking. These measurements are tabulated in table 1.

The curves (Figs. 2 and 3) for *Ribes glandulosum* and *R. rotundifolium* are parabolic in form; therefore slight accuracy can be expected in their extrapolation on regular cross-section paper. It can readily be shown, however, that such curves on regular cross-section paper must assume a linear form when the same data are plotted logarithmically. In figures 4 and 5 the measurements in table 1 have been plotted on logarithmic cross-section paper, forming straight lines which may be extrapolated with great accuracy.

TABLE 1.—*Measurements of width and corresponding area for Ribes rotundifolium and Ribes glandulosum*

R. glandulosum		*R. rotundifolium*	
Width in inches (Class centers)	Area in sq. inches (Average of 10 measurements)	Width in inches (Class centers)	Area in sq. inches (Average of 10 measurements)
1.0	0.64	0.6	0.31
1.1	0.76	0.7	0.38
1.2	0.84	0.8	0.52
1.3	0.93	0.9	0.62
1.4	1.22	1.0	0.71
1.5	1.43	1.1	0.88
1.6	1.43	1.2	0.98
1.7	1.61	1.3	1.14
1.8	1.90	1.4	1.31
1.9	2.27	1.5	1.63
2.0	2.41	1.6	1.72
2.1	2.63	1.7	1.93
2.2	2.90	1.8	2.08
2.3	3.26	1.9	2.24
2.4	3.38	2.0	2.56
2.5	3.83		
2.6	3.47		
2.7	4.49		
2.8	4.30		
2.9	4.75		
3.0	4.94		
3.1	5.93		
3.2	5.54		
3.3	6.08		
3.5	6.87		

Not only does logarithmic plotting simplify extrapolation, but it also permits a great reduction in the amount of data required for scale construction. As only two points are needed to determine a straight line, average area values need to be determined for only two values of width, one in the upper and the other in the lower ranges of leaf sizes. There is a possibility, however, of introducing rather large errors if the two points determined deviate from the straight line which would result were area measurements determined for intermediate widths in the range of leaf sizes. Therefore the two points should be determined from a much larger group of leaves than when each intermediate point is determined. Twenty-five measurements for both the upper and lower limits of width size classes would in all probability locate the straight line with as great accuracy as though ten leaves were measured in each tenth-of-an-inch width size class.

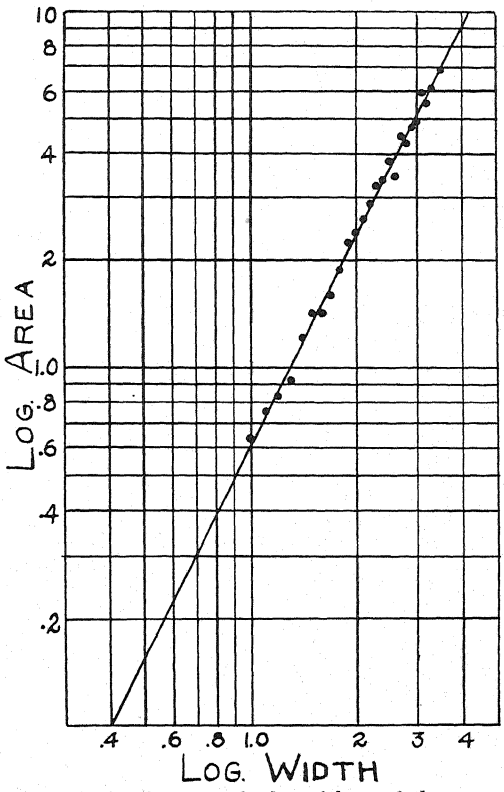


FIG. 4. Graph showing relation between the logarithms of the areas and the logarithms of the widths of leaves of *R. rotundifolium*.

The graduations of the scales shown in figures 1 are derived from the logarithmic graphs. Widths corresponding to each tenth of a square inch increment of area are laid off along a straight line. On the scale thus constructed the width graduations are marked with their corresponding area values. The area of any normal leaf of the species can then be determined as illustrated (Fig. 1).

The manner in which these leaf-area scales were constructed prevents them from being applied with accuracy to individual leaves or to short and non-representative series of leaves. If it is undesirable to use a planimeter in such cases, it is possible to increase the accuracy of the scale method by the construction of two scales instead of one, the second based upon another dimension of the leaf, such as length. Since irregularities in leaf shape frequently occur, as abnormal widths with subnormal lengths and vice versa, the area of an individual leaf may be closely approximated by averaging the readings of both scales.

The primary object in constructing the leaf area scales shown in figure 1 was to obtain instruments that would approximate within 10 per cent the area of large groups of leaves of each species of Ribes. Before putting the scales for *R. glandulosum* and for *R. rotundifolium* into general use, rough checks were obtained on their accuracy by comparing the scaled areas with the planimetered areas of new series of leaves.

The scale for *R. glandulosum* (Fig. 1) was checked by measuring the areas of 100 leaves taken at random from one large plant, by both the

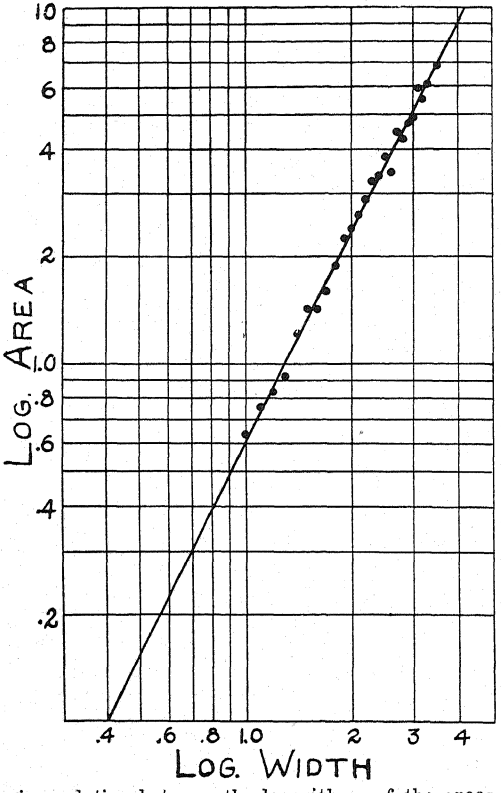


FIG. 5. Graph showing relation between the logarithms of the areas and the logarithms of the widths of leaves of *R. glandulosum*.

planimeter and scale methods applied independently by two individuals. The total area of the 100 leaves was found to be 275.6 sq. in. by both methods. In this check, 100 per cent relative accuracy is accidental and cannot be expected consistently.

The scale for *R. rotundifolium* (Fig. 1) was checked by measuring in like manner all leaves (a total of 605) not mutilated by insects from 10

plants. The total area of the 605 leaves measured by planimeter was 327.5 sq. in., and by the scale was 332.7 sq. in., a variation of 5.2 sq. in., or a plus error of 1.59 per cent by the scale method.

Judging from these somewhat meager tests, the two scales may be used with consistent accuracy greater than 95 per cent. However, the accuracy of any leaf-area scale will depend upon how well the species is represented in the sample of leaves from which the scale was constructed and in the sample being measured. Since a large sample of leaves is more likely to be representative of the species, it may be expected to furnish the more accurate results.

SUMMARY

This paper shows how to construct and test the accuracy of a scale for measuring the surface area of Ribes leaves. The scale reads directly in terms of area and gives results that are between 90 and 100 per cent accurate. A scale of greater accuracy can be constructed from a large series of leaf measurements. As the scale method of measuring the surface area of leaves is rapid, it will save much time in measuring a large number of leaves when an accuracy within 5 or 10 per cent is sufficient. This method should be valuable to phytopathologists and ecologists.

OFFICE OF BLISTER RUST CONTROL,
BUREAU OF PLANT INDUSTRY,
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ROOT-OBSERVATION BOXES

A. L. Dean[1]

Most of the ills to which the pineapple plant is heir—and it has rich legacies—are root troubles. Observation and study of growing roots in large numbers have become routine matters in our laboratories. This study of roots and their diseases has been greatly facilitated by the use of root-observation boxes. There is nothing especially new about the idea of using such boxes, but to use them so intensively and extensively in the study of roots is apparently unusual. With the thought that others may find them equally useful, this description is given.

Two types of boxes are being used. The small ones are modeled after those first described by Professor J. H. Comstock[2] in 1888 as "root cages." The large type was evolved here before we had any knowledge of the Comstock root cage. Both are useful, but for different purposes.

The small box is shown in figures 1 and 2. The exact dimensions are not matters of great importance; those given have proved convenient. These boxes are readily made in large numbers at very low cost. Briefly, they consist of a wooden frame comprising two ends and a bottom, with side strips top and bottom, and two glass sides. Wooden shutters are provided to keep the contents dark except at times of observation. With a table saw, the wooden pieces can be rapidly cut to size and saw scarfs run to receive the glass sides. A useful arrangement for removing the glass readily is indicated in figure 2. The upper side strips which hold the tops of the glasses in place are secured at one end by a single nail or screw and at the other by hook and eye. By unhooking the fastening and swinging the strip upward, the top of the glass is freed and the pane can be lifted out without disturbing roots or soil, and as readily replaced.

These boxes have found extensive use in biological field surveys of pineapple soils. Large numbers of soil samples have been placed in these boxes and pineapple slips or crowns set in them, as indicated in figure 1, and the bozes placed in the glass-house. In a short time the roots start out and numbers of them grow along the glass sides. Every few days the boxes are examined for signs of root injury. Examination is readily made by laying each in turn in an almost horizontal position on a stand built for the purpose, removing the shutters, and examining the roots through the glass, first

[1] Director, Experiment Station of the Association of Hawaiian Pineapple Canners, University of Hawaii, Honolulu, Hawaii.

[2] Cornell University. Bulletin of the Agricultural Experiment Station III; November, 1888.

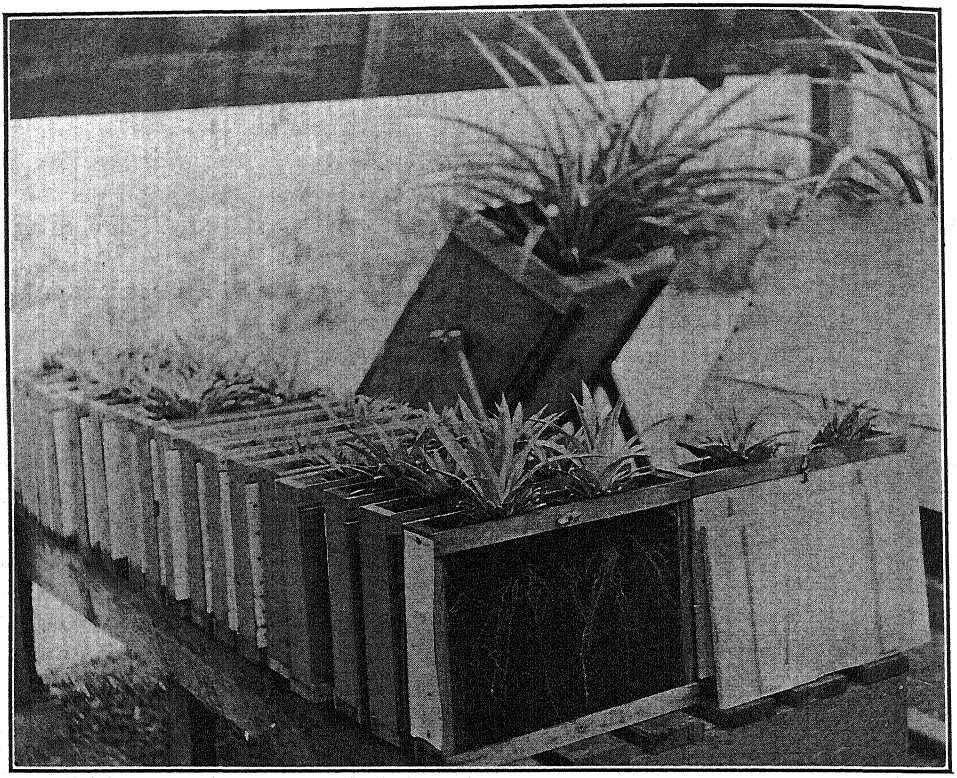


FIG. 1. Small root-observation boxes; a large box in the background.

with the unaided eye and then with the binocular. By examining both sides of a box a large number of roots is inspected and signs of injury readily detected. If portions of diseased roots are wanted for intensive study, they can readily be secured by carefully lifting the glass and cutting out desired portions. Soil insects may be observed at work, nematode injury followed, and the incidence and progress of fungus attacks noted. A good qualitative, and a rough quantitative, parasite analysis of a soil sample is thus secured. Parasites which would have wholly escaped us by the usual method of pulling up sickly plants in the field have been discovered at work and their progress observed in detail.

Studies of the life histories of various root parasites and of the extent and nature of the damage done have been facilitated. In a large steam sterilizer the boxes, with soil in place, are readily sterilized. Clean, healthy plants set in such sterile containers and soil will, when roots have started out, be in ideal condition for inoculation and subsequent study. By care-

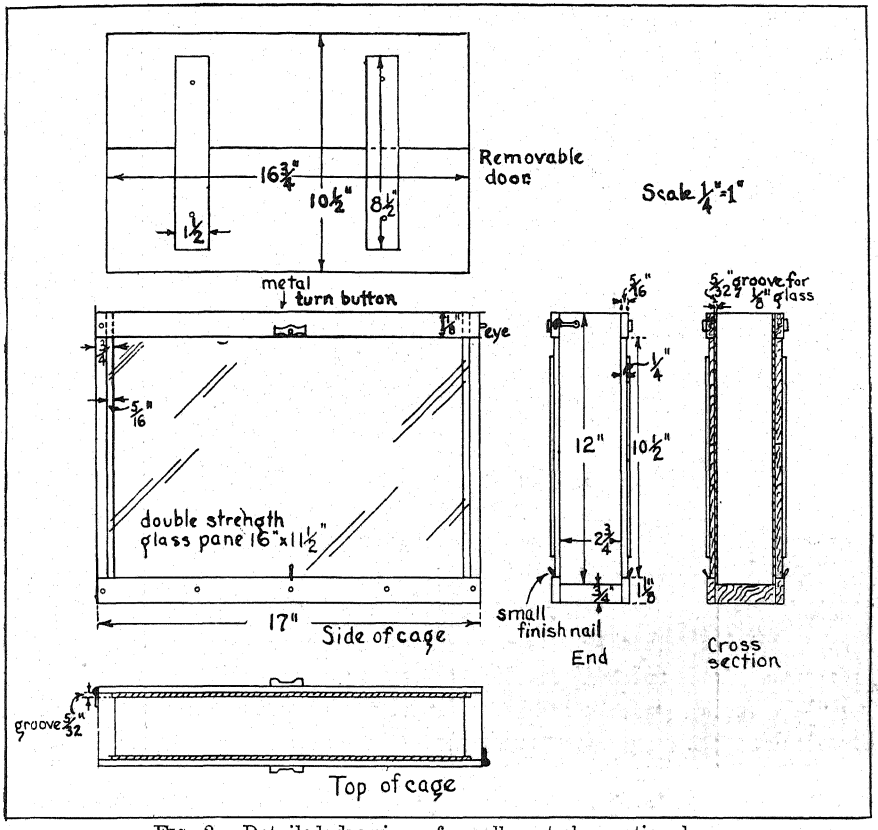


FIG. 2. Detailed drawing of small root-observation box.

fully removing the glass the inoculum may be placed in any desired position relative to the roots, the glass replaced, and observations made on the course of events. Effects of chemicals, temperature, moisture, etc., on host and parasite are easily observed.

It is obvious that physiological studies involving observations of roots are facilitated by the use of such glass-sided boxes. We have cemented strips of glass into these boxes with battery wax in such a manner that the space inside was divided into two portions by this glass partition reaching almost to the top. A plant growing astride of this partition sends part of its roots into each compartment, where different conditions may be provided.

The large type of box was developed here before we had become acquainted with the Comstock root cage. These boxes are shown in figures 3 and 4. They are adapted to studies involving more prolonged growth and

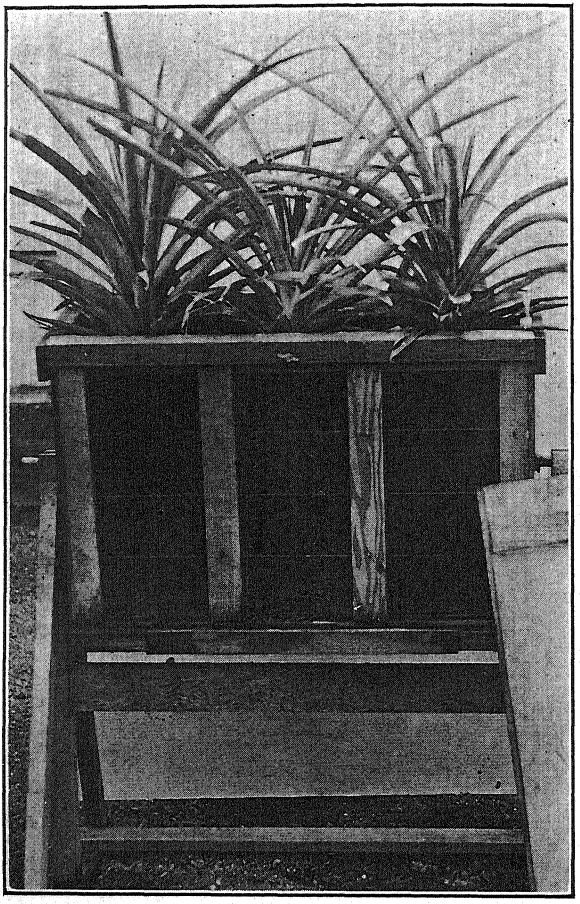


FIG. 3. Large root-observation box with shutter removed.

greater development. These larger boxes are provided with a number of panes of glass held in place by wooden strips in such a manner that the panes can be removed if desired. Wooden shutters are provided as in the small boxes. Our first boxes were made to stand on a bench. This was not convenient, however, when one wished to use the binocular microscope. As now made, the boxes have a ¾-inch pipe flange attached to each end and a short piece of pipe screwed into each. These pieces of pipe rest in slots in the supporting frame, allowing the boxes to swing. In this way the box may be swung up into a horizontal position and held there by a hook; or a stick can be used to support it if desired. The binocular can then be

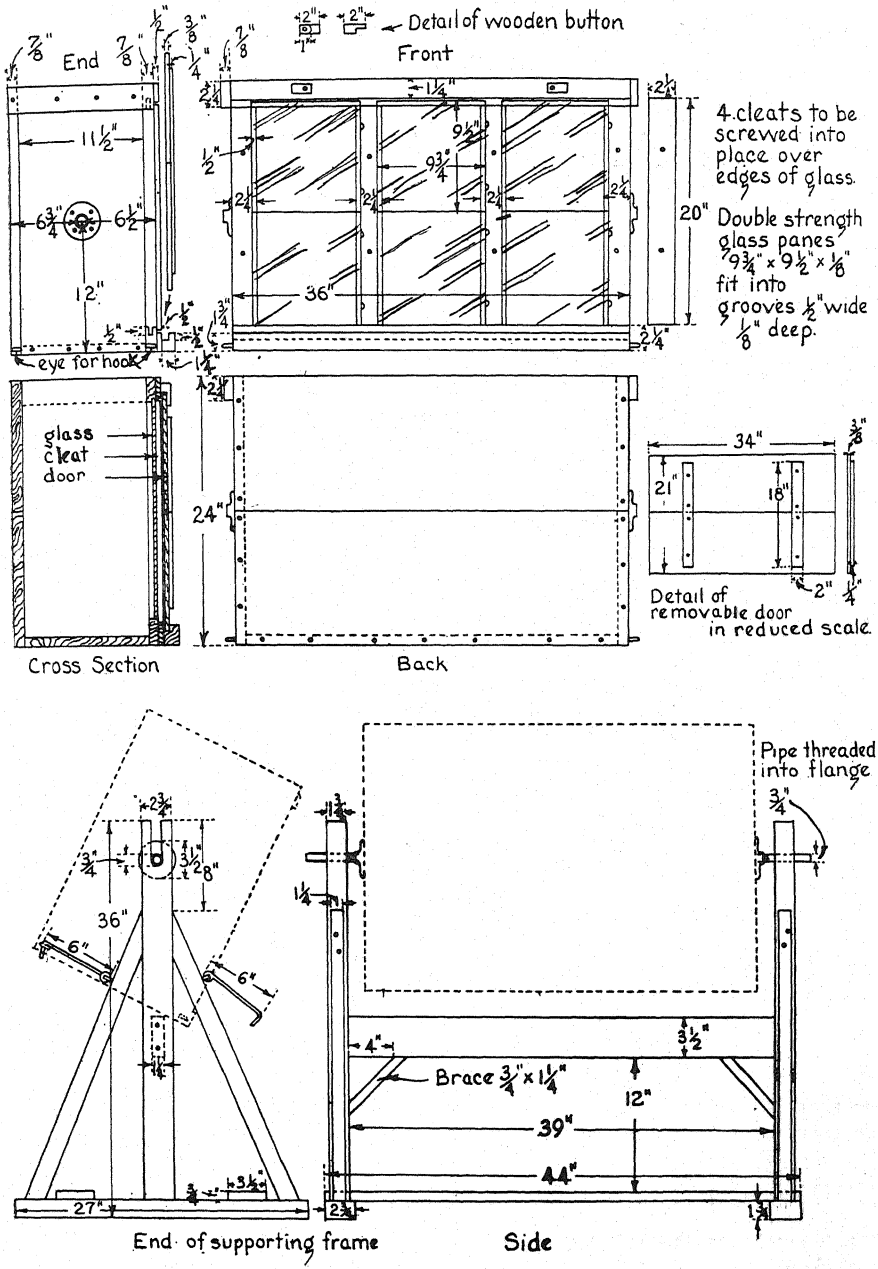


FIG. 4. Detailed drawing of large, swinging root-observation box.

used in comfort and the roots thoroughly studied. In order to encourage roots to grow next to the glass the boxes are usually held in a slightly tilted position to take advantage of the geotropic response.

EXPERIMENT STATION,
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TITLES AND ABSTRACTS OF PAPERS READ AT THE TENTH ANNUAL MEETING OF THE CANADIAN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, GUELPH, ONTARIO. DECEMBER 20 AND 21, 1928

Further experiments with millet smut.—R. E. STONE.

The spur blight of raspberries.—L. W. KOCH.

Notes on the fireblight situation.—D. H. JONES

Acid sterilization of liquid culture media.—B. G. MONTSERIN and J. G. COULSON.

President's address—Retrospect and Prospect.—D. L. BAILEY.

A preliminary report of the results of some field tests in the use of seed disinfectants for the prevention of loose smut of barley.—J. E. HOWITT and R. E. STONE.

The European corn borer.—L. CAESAR.

Studies in the physiology and cytology of Ustilago zeae and Sorosporium reilianum.—W. F. HANNA.

Mosaic diseases of plants.—G. P. CLINTON.

The relation of wound gum to fungous invasion in peach and grape.—R. S. WILLISON.

The new willow disease of Canada and New England.—G. P. CLINTON.

Black mould of onions.—J. E. MACHACEK.

Observations on the relative resistance to smut infection of some varieties of oats.—R. E. STONE.

A severe outbreak of bacterial wilt of cruciferae.—D. H. JONES

Observations on needle blight of white pine.—J. DEARNESS.

Bulb growing in Holland in its relation to disease control.—F. L. DRAYTON.

The subject is introduced with a description of the events which led up to the three months' visit to Holland in the summer of 1928 at the invitation of the Netherlands Government. An outline is given of the location of the bulb-growing districts, the research and educational institutions dealing with various phases of bulb culture, and the various associations in connection with bulb culture. The exceedingly specialized and intensive nature of successful commercial bulb culture is stressed.

The main subject matter is divided into five sections as follows: General Bulb Culture, Hyacinth, Narcissus, Tulip, and Gladiolus. In the first of these the general year-round routine is described, emphasizing the procedures related to disease control.

Under the other headings the points of divergence from the general culture, as related to the individual bulb crops, and the diseases to be combatted in each are described.

Woodgate rust in Canada.—A. W. MCCALLUM.

The gall-forming rust on Scotch pine reported, under the above name, in 1925 from New York has been found to occur in Canada. It was first collected in 1918 but at that time was believed to be *Cronartium cerebrum*. Apart from Ottawa it has been found in Ontario at St. Williams in Norfolk County and at Orono in Durham County, and in Quebec at Berthierville and probably also at Lachute. Specimens have been received from Nappan, Nova Scotia. Excepting at Ottawa, no galls more than seven years old were found. At Ottawa there are galls which are approximately forty years of age.

Histological studies of wheat infected with Ophiobolus graminis Sacc.—R. C. RUSSELL.

Penetration usually takes place directly through the outer walls of epidermal cells, without the formation of appressoria and without relation to the position of the tangential walls. External mycelium, spreading over the surface of the coleoptile, gives off short, blunt branches which develop minute "penetration pegs." The latter average about 0.6 μ in diameter. Certain stains color the outer walls of epidermal cells immediately around the points of penetration. The host-cell walls tend to swell around these points and the swollen walls stain deeply with acid fuchsin or safranin. Penetration points are sometimes exceedingly numerous.

Host cells build up conical structures, callosities, around invading hyphae. Hyphae which succeed in emerging from the tips of callosities swell to their normal diameter and proceed through the host cell. Callosities may or may not be formed as the hyphae penetrate from cell to cell. Callosities and thickenings of cell walls sometimes occur to a depth of several cells in the tissues of the coleoptile. The parenchymatous tissue breaks down more rapidly than the fibro-vascular bundles under the action of the fungus. The tissues of the roots, crown, and sub-crown internode are invaded and destroyed.

The biology of Fomes pinicola (Sw.) Cooke.—IRENE MOUNCE.

A list of 89 hosts for *Fomes pinicola* has been compiled. Spore germination, cultural characters of mycelium from various sources, formation of a line of demarcation in mixed cultures, and heterothallism have been studied.

Cultures varied in rate of growth, color production, and texture of the mycelial mat. These differences have been ascribed to individual variation rather than to host influence. Sporophores were obtained frequently, one producing three definite pore-layers. The context of such sporophores was typical both in color and texture, and the spores produced were viable.

Fomes pinicola is heterothallic and bisexual. Monosporous mycelia did not change spontaneously from the haploid to the diploid condition. Many so-called sexual strains, which were completely cross fertile, were found. Monosporous mycelia from deciduous hosts were mutually fertile with those from coniferous hosts; strains from France and Sweden were mutually fertile with all Canadian strains except one. Judged by the clamp-connection criterion for the identity of species these results furnish experimental evidence in support of the general belief that (1) *Fomes marginatus* and *Fomes pinicola* are one and the same species, and (2) that European and Canadian collections of this fungus are identical.

PHYTOPATHOLOGY

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STUDIES IN THE PHYSIOLOGY AND CYTOLOGY OF USTILAGO ZEAE AND SOROSPORIUM REILIANUM[1]

W. F. HANNA

INTRODUCTION

Two smuts of corn are known to occur in the United States. The one of greater importance from the standpoint of distribution and loss is caused by *Ustilago zeae* (Bekm.) Ung. This fungus, widely distributed in the great corn-growing region of the Middle West, may infect the actively growing tissues of the corn plant at any stage in the plant's growth. The conspicuous smut galls, which appear at or near points of infection, may produce stunting of the plants, and, if the female inflorescence is involved, the ears may fail to develop. The other smut is caused by *Sorosporium reilianum* (Kühn) McAlpine. In the Pacific-Coast regions this fungus is found commonly on corn, but in other parts of the United States it is confined chiefly to sorghum. In Australia, however, corn smut is caused almost entirely by *S. reilianum*. It is generally considered that plants are infected by this fungus in the seedling stage. From then on, the growth of the parasite is systemic in the tissues of the host plant, and, at maturity, the inflorescence generally becomes a powdery mass of chlamydospores. It is of interest that Reed, Swabey, and Kolk (30) have found recently that *S. reilianum* is composed of two physiologic strains, one of which occurs on corn and the other on sorghum.

HISTORICAL REVIEW

The working out of the life histories of the smuts has contributed one of the most interesting chapters in the development of plant pathology. A number of early investigators had observed that in certain smuts cell fusions occurred frequently. In some species these fusions take place between neighboring sporidia; in others, between two cells of the promycelium. De

[1] Published with the approval of the Director as paper No. 822 of the Journal series of the Minnesota Agricultural Experiment Station.

This investigation was carried out under the direction of Dr. E. C. Stakman, whose assistance and advice are gratefully asknowledged. The writer also wishes to thank Dr. J. J. Christensen, Dr. H. P. Barss, and Professor Pantanelli for providing collections of chlamydospores.

Bary pointed out the similarity between these unions and the sexual fusions which occur in the Conjugateae, and suggested that the cell fusions in the smuts might represent a reduced sexual process. Brefeld firmly opposed this interpretation. He maintained that these cell fusions were merely vegetative unions and that the smuts were entirely lacking in any form of sexual reproduction.

The first extensive investigation of the nuclear phenomena in the life history of the smut fungi was made by Dangeard (11). He studied a number of species belonging to the genera Entyloma, Tilletia, Urocystis, and Ustilago, and found the chlamydospores to be uninucleate and, on germination, to produce promycelia, each cell of which contained a single nucleus. If sporidia budded from the promycelium, they, too, were uninucleate. The young chlamydospores produced in the host plant were binucleate but, as they matured, the two nuclei fused so that each ripe chlamydospore contained but a single nucleus. This nuclear fusion in the chlamydospore was regarded by Dangeard as a reduced sexual process. Harper (19), working with *Ustilago maydis, U. antherarum,* and *U. scabiosa,* confirmed Dangeard's observations regarding the fusion of the two nuclei in the chlamydospore.

Harper had investigated the fusions between sporidia and those between promycelial cells, but he observed no migration of protoplasm from one cell to the other, and the nuclei appeared to remain in their respective cells throughout the entire process. However, in 1910, Lutman (27) found that, when the promycelial cells of *U. hordei, U. avenae,* and *U. tritici* conjugate in pairs, the nucleus from one cell passes over into the other cell, where the two nuclei come to lie side by side. He examined the tissue of plants infected with *U. levis* and *U. maydis,* respectively, and found the mycelium of both smuts to be made up of multinucleate cells. Immediately before spore formation began, certain cells appeared to contain each a pair of nuclei which later fused, so that the mature spore contained but a single nucleus. The possible relationship between the nuclei which become associated during the fusion of two sporidia or of two promycelial cells and the pair of nuclei which finally fuse in the chlamydospore appears to have been overlooked by Lutman, for he states: "In the smuts the morphological equivalents of the oogone and the antherids are the fused conidial or promycelial cells. Functionally the fusion of the cells is no longer of much importance in the life cycle of the smut but they still represent the primitive gametes. In the Basidiomycetes cell fusion has disappeared entirely but in the smuts it is retained in a rudimentary state and is only functional in a limited fashion under certain conditions."

In 1912, Rawitscher (29) investigated the life histories of *U. carbo* and *U. maydis.* When the spores of *U. carbo* germinated, fusions were observed

to take place either between the promycelial cells or between the sporidia produced from cells which had not fused. After fusion, the nucleus from one cell passed over to the other cell. The mycelium in the tissues of the host plant was found to consist of binucleate cells, and not multinucleate cells, as had been observed by Lutman. At the time of spore formation, the pair of nuclei in each cell fused, thus giving rise to uninucleate chlamydospores. In *U. maydis,* no fusions were observed to take place between promycelial cells or between sporidia. The cells of the mycelium in the host plant were uninucleate and, immediately before spore formation, these cells appeared to fuse in pairs, the nucleus from one cell passing into the neighboring cell. The resulting binucleate cell rounded itself off and the two nuclei fused, thus producing a uninucleate chlamydospore. As a result of his observations, Rawitscher (29) concluded that the two nuclei which eventually unite in the chlamydospore of *U. carbo* are the direct descendants of the two nuclei which become associated during the fusion of two sporidia or two promycelial cells. In *U. maydis,* the cycle of events was believed to be essentially the same as in *U. carbo,* with the important difference, however, that in the former smut no sporidial fusions occur, the association of nuclei taking place only at the time of spore formation. Because of this delayed nuclear association, almost the complete life cycle of the smut appeared to be passed in the haploid condition.

Rawitscher found no clamp connections on the parasitic mycelium of *U. maydis,* but Seyfert (32), in a recent paper, figures numerous clamp connections and also shows that the binucleate condition arises some time before spore formation.

A number of investigators had observed that sporidial fusions occur in certain smuts, but Kniep (22) was the first to show that these fusions take place only between certain sporidia. In *U. violacea,* he demonstrated the existence of two kinds of sporidia, A and B, present in approximately equal numbers. Fusions occurred only when A sporidia and B sporidia were brought together. Kniep suggested that the physiological differences in the sporidia were determined by sex factors and that during the nuclear divisions accompanying spore germination, a segregation of these factors took place resulting in the production of two kinds of sporidia of opposite sex.

In the smut fungi, therefore, a well-defined alternation of generations seems to occur. The mature chlamydospore contains a single diploid nucleus. During the nuclear divisions which accompany the germination of the spore, a reduction takes place, with the result that each promycelial cell and each sporidium receives a single haploid nucleus. In some species, the binucleate condition is then initiated by the conjugation of sporidia or of promycelial cells; in others, *e.g., U. zeae,* by the conjugation of neighboring cells of the mycelium within the host plant. This binucleate condition per-

sists until immediately before spore formation when the two nuclei in each cell fuse and chlamydospores once more are formed, each containing a single diploid nucleus.

USTILAGO ZEAE

Earlier investigations

Brefeld (5) made a careful study of spore germination in *U. zeae* and showed how the germ tubes from the sporidia infect the corn plant. He believed that the chlamydospores borne in the smut galls germinated in the soil and produced sporidia which, in turn, were able to multiply by a process of yeast-like budding. These sporidia were then carried off by the wind, and, settling upon the growing tissues of the corn plant, sent out germ tubes which caused infection by direct penetration of the epidermis.

At the time when Brefeld made his experiments, very little was known of the nuclear phenomena which occur during the life history of the smuts. More recently, however, Rawitscher (29) established the fact that in *U. zeae* an alternation takes place between the haploid and the diploid condition. He believed the haploid condition to extend from the time of chlamydospore germination to a period immediately before spore formation. The diploid phase, therefore, was restricted to a single cell which, at maturity, became the chlamydospore. This would mean that almost the complete life cycle of *U. zeae,* including the parasitic stage, is passed in the haploid condition.

The work of Stakman and Christensen (34) demonstrated that in all probability *U. zeae* is heterothallic. The cultures which they employed in their experiments originated from a number of chlamydospores, but they were grown separately for periods ranging from one to three years and, at the time the inoculations were made, each culture appeared to be pure as to type of growth. They found that these cultures produced no galls when injected singly into corn plants, but when paired together in certain combinations they produced an abundance of galls. It was concluded, therefore, that *U. zeae* is made up of at least two sexual strains, and that gall formation takes place only when these two strains are brought together in the host plant.

Outline of investigations

The present investigation is a continuation of the work of Stakman and Christensen. The main points to be investigated were: (1) The behavior of the nuclei during spore germination; (2) the capacity of monosporidial cultures to produce galls on injured and uninjured plants; (3) the capacity of various combinations of monosporidial cultures to produce galls on injured and uninjured plants; (4) the behavior of monosporidial cultures from chlamydospores collected in different districts when paired together in the host plant; and (5) the period at which hyphal fusion and pairing of the nuclei occur in the host plant.

Spore germination

Germination of the spores of *U. zeae* has been figured by Brefeld (5), Hitchcock and Norton (20), Clinton (9), and Stakman (33). Maire (28) gave illustrations showing the nuclei of the budding sporidia. However, the writer has been able to find only two figures showing the nuclei during spore germination; they are given in a paper by Istvanffi (21), published in 1895.

Spores germinate readily on 1 per cent malt agar. In order to obtain material for a study of the nuclei during germination, a thin film of this medium was spread on one side of a glass slide and the spores were dusted on the surface of the film with a small camel's-hair brush. The slides were then inverted over glass rods in a petri dish containing a few drops of distilled water. When the desired stage of germination had been reached, the material was fixed by adding a few drops of Flemming's weaker solution which was allowed to act for about 15 minutes. The fixative was then removed by carefully adding a few drops of distilled water and sucking up the excess liquid with filter-paper. In order to prevent the spores from being washed off during the process of staining, the slides were passed first into a bath of ether, and then into a 0.2 per cent solution of collodion made up with equal parts of alcohol and ether, as suggested by Kniep (24). The thin film of collodion left on the slide prevented the spores from being washed off during subsequent manipulations. The material was stained in haematoxylin (Heidenhain's method), given a short counter stain in erythrosin, and finally mounted in balsam.

The various stages of spore germination and the budding of the sporidia are shown in Plate VII, 1–19. The first division of the fusion nucleus takes place in the chlamydospore. When the promycelium has been produced, one of the daughter nuclei migrates into it, while the other one remains in the spore. The two nuclei then undergo a second division and one of the nuclei from the spore moves up into the base of the promycelium, thus giving three nuclei to the promycelium and one to the spore. Three septa are generally formed, separating the nuclei from one another, but sometimes it is difficult to see them, even in stained preparations. The sporidia usually appear at definite positions on the promycelium—at the tip, at the upper ends of the central cells, and at the junction between the promycelium and the spore. Before the sporidium has reached full size, the nucleus in the promycelial cell divides, and one daughter nucleus migrates into the sporidium while the other one remains in the cell. In this way, a promycelium is able to produce several successive generations of sporidia. By using the spore isolator (18), three generations of sporidia were removed from a promycelium and a fourth generation was seen to develop a short

time afterwards. This process of sporidial production is illustrated in Plate VII, 15.

The sporidia generally are uninucleate. However, some sporidia may have two or more nuclei. On nutrient media, they reproduce by budding. The behavior of the nuclei in this process is shown in Plate VII, 16–19.

The Effect of Injecting Corn Plants with Individual Monosporidial Cultures and with Various Combinations of these Cultures

Cultures were made from four different collections of chlamydospores. Three of these collections, A, B, and C, were obtained from corn grown on plots at University Farm, St. Paul. The third, D, was sent to Dr. Stakman by Professor Pantanelli, of Bari, Italy. Single chlamydospores were placed in hanging drops of 1 per cent malt agar and, when they had germinated, the sporidia were removed from the promycelia and propagated separately. The method of isolating the individual sporidia has been described in a separate paper (18). Inoculum for the infection experiments was obtained by transferring each monosporidial culture to an Erlenmeyer flask containing a nutrient solution composed of 1 per cent malt extract and 2 per cent dextrose. In this solution the sporidia multiplied very rapidly.

Corn plants of the variety Golden Bantam were used in all of the experiments. They were grown in pots in the greenhouse, each pot having eight plants. The plants were inoculated when they were about four weeks of age, the nutrient solution containing the sporidia being injected into the growing points of the stems by means of a hypodermic syringe. When plants were to be inoculated with a combination of two monosporidial cultures, the mixing of the cultures was performed in a sterile Erlenmeyer flask immediately before inoculation. One pot of eight plants was used for each inoculation, and all experiments were made in duplicate. Plants inoculated with sterile nutrient solution were employed as checks.

Sporidia were isolated from seven chlamydospores. Two of these chlamydospores were taken from collection A, three were from collection C, and one was taken from each of the collections B and D. Where possible, a record was kept of the position on the promycelium occupied by each sporidium, and for convenience the sporidia were numbered 1, 2, 3, and 4, from the tip of the promycelium to the spore. A number was also given to the chlamydospore from each collection. Thus, A21 refers to the sporidium at the tip of the promycelium produced from spore number 2 of collection A. In the case of spores C1 and C3, the four sporidia were isolated from each promycelium but it was not possible to determine the relative positions which they occupied. The numbers which have been given to these sporidia, therefore, do not refer to the positions which they occupied on the promycelia.

The results of inoculating corn plants with monosporidial cultures used singly and also combined in various ways are presented in tabular form. The sign (+) indicates that the inoculated plants produced galls; the sign (–) indicates that no galls appeared.

It is of interest to note, first of all, that whereas certain cultures when combined together produced galls, none of the 28 monosporidial cultures when injected alone was capable of inducing gall formation. This result confirms the conclusion of Stakman and Christensen that *U. zeae* is heterothallic and shows that galls are produced only when plants are infected by two cultures originating from sporidia of different sex.

All of the possible combinations were made between cultures of the four sporidia from each of the eight chlamydospores. On the basis of these reactions, it is possible to divide the chlamydospores into three groups: (1) Those which produced two sporidia of one sexual group and two of the opposite sexual group; (2) those which produced sporidia of four sexual groups; (3) those which produced three sporidia of one sexual group and one of the opposite group.

The production of sporidia or promycelial cells belonging to two sexual groups seems to be quite common in the smuts. This condition, first reported by Kniep (22) in *Ustilago violacea,* has since been observed in several other species. In *U. zeae,* five of the seven chlamydospores investigated produced promycelia bearing two sporidia of one sex and two of the opposite sex. However, the sporidia were found to be arranged on the promycelia in two different ways. The sporidia from spores C2 and D1 were grouped so that numbers 1 and 2 were of one sex and 3 and 4 were of the opposite sex. Table 1, giving the reactions of the sporidia from spore D1, illustrates this condition. The arrangement of the sporidia from spores

		AB		ab	
		1	2	3	4
AB	1	–	–	+	+
	2	–	–	+	+
ab	3	+	+	–	–
	4	+	+	–	–

TABLE 1.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae.* Cultures of the four sporidia from chlamydospore D1 (Italy) were used singly and in pairs.

		AB	ab	AB	ab
		1	2	3	4
AB	1	–	+	–	+
ab	2	+	–	+	–
AB	3	–	+	–	+
ab	4	+	–	+	–

TABLE 2.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae.* Cultures of the four sporidia from chlamydospore B1 (Minnesota) were used singly and in pairs.

A2 and B1 was such that numbers 1 and 3 were of one sex and 2 and 4 were of the opposite sex. This condition is illustrated by the reactions of the sporidia from spore B1, as shown in table 2. Spore C3 produced two sporidia of one sex and two of the opposite sex, but the positions of the sporidia on the promycelium were not recorded.

It has been shown (17) that some of the basidia of *Coprinus lagopus* may bear spores belonging to four sexual groups. The genetic constitution of these spores is such that when their mycelia are paired in all possible ways only two combinations result in diploid mycelia having clamp connections. Up to the present, a condition analogous with this has not been reported in the smut fungi. In *U. zeae,* however, a single promycelium may produce sporidia of four different kinds, as is shown in table 4. This type of sporidial production was found only in chlamydospore A1. When cultures of the four sporidia from this chlamydospore were paired in all possible ways and injected into corn plants, it was found that galls were produced by the combination of sporidia 1 and 3, and by 2 and 4, but by no others.

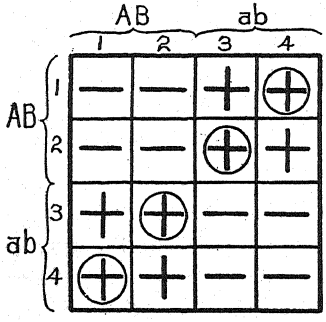


TABLE 3.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae.* Cultures of the four sporidia from chlamydospore C2 (Minnesota) were used singly and in pairs.

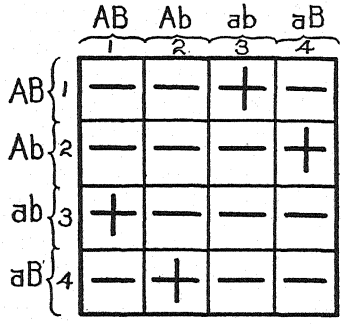


TABLE 4.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae.* Cultures of the four sporidia from chlamydospore A1 (Minnesota) were used singly and in pairs.

The reactions of the sporidia from spore C2 suggest a condition intermediate between the bisexual type of spore D1 and the so-called quadrisexual type of spore A1. As is shown in table 3, the sporidia from this spore fall into two groups, 1 and 2 being of one sex and 3 and 4 of the opposite sex. However, two of the matings—1 with 4, and 2 with 3—produced only a few small galls. In the table, these weak reactions have been denoted by circles about the (+) signs. A comparison of tables 3 and 4 will show that, had sporidia 1 and 4, and 2 and 3, of table 3, failed to produce any galls, a grouping identical with that in table 4 would have been obtained.

On the other hand, had these matings produced the normal number of galls, table 3 would have been identical with table 1.

The reactions of the sporidia from spore C1 are presented in table 5. These sporidia belong to two sexual groups, but instead of occurring in pairs, this tetrad has three sporidia of one sex and only one of the opposite sex. Unfortunately, the order in which they occurred on the promycelium is not known, but it is probable that the nucleus of one of the promycelial cells degenerated and that one of the others produced two sporidia. A condition suggesting this may be seen in Plate VII, 12, where the promycelial cell immediately above the chlamydospore appears to be producing sporidia from two places.

	AB 1	AB 2	ab 3	AB 4
AB 1	—	—	+	—
AB 2	—	—	+	—
ab 3	+	+	—	+
AB 4	—	—	+	—

TABLE 5.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae*. Cultures of the four sporidia from chlamydospore C1 (Minnesota) were used singly and in pairs. (Relative positions of the sporidia on the promycelium were not determined.)

		AB D11	AB D12	ab D13	ab D14
ab	C31	+	+	—	—
ab	C32	+	+	—	—
AB	C33	—	—	+	+
AB	C34	—	—	+	+

TABLE 6.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae*. Cultures of the four sporidia from chlamydospore D1 (Italy) were paired with cultures from the four sporidia of chlamydospore C3 (Minnesota).

Some species of the Hymenomycetes, *e.g., Schizophyllum commune* (25) and *Coprinus lagopus* (17), have been shown to be composed of distinct geographical strains and, when monosporous mycelia from two strains are paired together, all of the matings produce diploid mycelia bearing clamp connections. Vandendries (35) has shown recently that certain strains of *Coprinus micaceus* exhibit a high degree of intersterility. A few matings were made between monosporidial cultures of *U. zeae* obtained from different collections of chlamydospores, but no evidence of complete interfertility or intersterility was obtained. The four monosporidial cultures from spore C3 of the Minnesota collection reacted in a perfectly normal manner with the four monosporidial cultures of spore D1 from Italy. The results of these matings, given in table 6, show that the two sexual groups of the Minnesota cultures are identical with those of the Italian cultures.

Reduction and Segregation during Spore Germination

In certain respects the fungi are particularly suitable objects for a study of the inheritance of Mendelian characters. Many species complete their life cycles in a relatively short time and, unlike the higher plants, the haplonts may be kept in culture and used for any number of experiments. In spite of this fact comparatively little work has been done on the genetics of the fungi. Probably the most extensive investigations have been made in connection with the reactions of monosporous mycelia of some of the Basidiomycetes and Ascomycetes. Kniep (25) and others have interpreted their results in terms of "sex factors," while Brunswick (6) has employed the term "sterility factors." By using the latter term the reactions of these fungi are brought in line with somewhat similar phenomena in some of the higher plants; but, whichever term is employed, the genetic interpretation of the results is the same.

A relatively large number of matings would be required in order to make a thorough study of the factors determining the reactions of monosporidial cultures of *U. zeae*. However, with the data available, some interesting possibilities are suggested.

The reactions of the four sporidia from spore A1 (table 4) can be explained best by assuming that two pairs of factors—(Aa) and (Bb)—are involved and, furthermore, that these factors are borne in different chromosomes. The fusion nucleus of the chlamydospore, therefore, must possess the two pairs of homologous chromosomes bearing the factors (Aa Bb). When the spore germinates, the nucleus divides twice and segregation of these factors takes place so that each nucleus of the promycelium receives one factor from each pair. Each sporidium, in turn, will receive a single haploid nucleus from its promycelial cell. The genetic constitution of the sporidia will depend upon the time of chromosome reduction and also upon the manner in which segregation has occurred. A reduction of both pairs of chromosomes in the first division would result in the promycelium bearing two sporidia of one sex and two of the opposite sex. According to this method of segregation, the sporidia might be represented as (AB), (AB), (ab), (ab), or (Ab), (Ab), (aB), (aB). Obviously, this arrangement would not explain the reactions of the sporidia from spore A1. A reduction of both pairs of chromosomes in the second division might produce sporidia of two kinds, or of four kinds, according to the manner in which segregation took place. Of the promycelia bearing sporidia of two kinds, some might have two sporidia with the factors (AB) and two with the factors (ab); others might have two sporidia with the factors (Ab) and two with the factors (aB). Furthermore, it would be possible for the sporidia on each of the two types of promycelia to be arranged in any

one of four different ways. All of the eight possible arrangements would be different from those which might occur as a result of reduction in the first nuclear division. If the sporidia were of four kinds—(AB), (ab), (Ab), (aB)—they might be arranged also in eight possible ways. The sporidia from spore A1 are all different, since pairing occurred only between numbers 1 and 3 and numbers 2 and 4; but the order in which they were borne on the promycelium does not agree with any of the eight possible arrangements which might result from the reduction of both pairs of chromosomes in the second division. The only other possibility is that one pair of chromosomes separated in the first division and the other pair in the second division. This type of segregation is illustrated in figure 1. Such a scheme would result in the production of four kinds of sporidia so

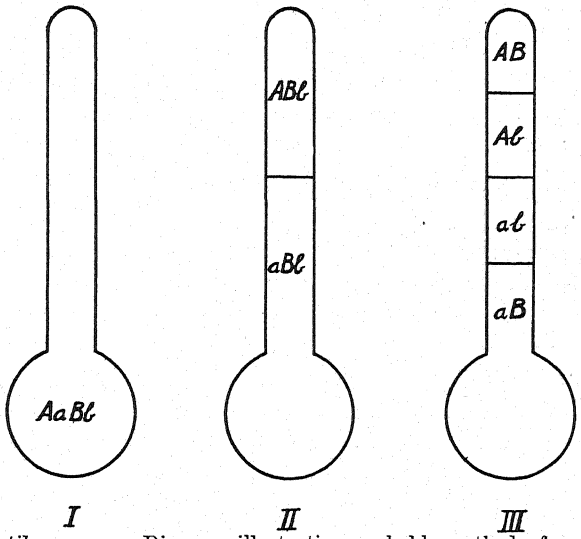


FIG. 1.—*Ustilago zeae.* Diagram illustrating probable method of segregation in the promycelium of spore A1. I: The fusion nucleus of the spore at time of germination possesses both pairs of factors. II: Segregation of the factors (Aa) occurs at the first nuclear division. III: Factors (Bb) segregate at the second division.

arranged that reactions would take place only between numbers 1 and 3 and numbers 2 and 4. As the sporidia of spore A1 were arranged on the promycelium in this order, it seems reasonable to conclude that one pair of homologous chromosomes separated in the first division and that the other pair separated in the second division.

Spores C2 and D1 produced sporidia of two kinds only, numbers 1 and 2 being of one sex and numbers 3 and 4 of the opposite sex, so that segregation of both pairs of factors must have taken place during the first division of

the fusion nucleus. This paired arrangement of the sporidia is illustrated in table 1. Two other spores—A2 and B1—also produced sporidia of two kinds only, but instead of being arranged in pairs on the promycelium, the sporidia of the same sex occurred alternately, numbers 1 and 3 being of one sex and numbers 2 and 4 of the opposite sex. This condition is shown in table 2. Such an arrangement could come about only by a segregation of both pairs of factors during the second division of the fusion nucleus.

Although the sporidia of spore B1 belong to only two sexual groups, they may be classified further on the basis of their cultural characters. When the sporidia had been removed from the promycelium and were growing in hanging drops of nutrient agar, it was seen that numbers 1 and 2 produced an abundance of sporidia and that numbers 3 and 4 produced a mycelial type of growth with relatively few sporidia. These morphological differences were even more pronounced when the cultures were transferred to Erlenmeyer flasks containing potato-dextrose agar. The appearance of the cultures on this medium is shown in figure 2. Sporidia 1 and 2 from the

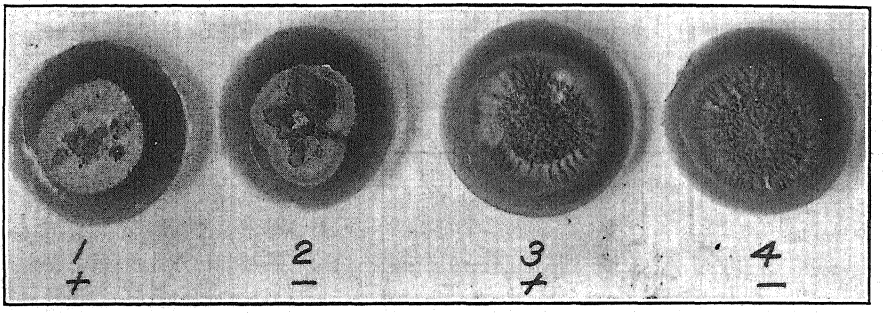


FIG. 2.—*Ustilago zeae.* Cultures of the four sporidia from spore B1 on potato-dextrose agar. Colonies 1 and 2 consist of sporidia; colonies 3 and 4 of a reddish-brown mycelium. The sporidia are numbered, according to position, from the tip of the promycelium to the chlamydospore.

two terminal cells of the promycelium gave white, yeast-like colonies, whereas, sporidia 3 and 4 from the two lower cells produced a reddish-brown mycelial growth. Therefore, although segregation of the sex factors must have taken place during the second nuclear division, the factors determining color and type of growth must have segregated with the first division of the fusion nucleus. This indicates also that the sexual reaction of a culture is not necessarily linked with a particular type of growth on artificial media.

From the evidence just presented, it would appear that in *U. zeae* a segregation of factors may take place during either the first or the second division of the fusion nucleus. Thus, in two spores (C2 and D1) segrega-

tion was completed at the first division; in one (A2) at the second division; while in two spores (A1 and B1) there is evidence of segregation at both divisions. Although botanists generally have favored the idea of pre-reduction, there is good reason to believe that, in both plants and animals, segregation may take place at either the first or the second division of the fusion nucleus. In the fungi, evidence in support of segregation at the second division was advanced by Funke (16) for *Hypholoma fasciculare* and *Collybia velutipes* and by the writer (17) for *Coprinus lagopus*. Allen (1) found that in *Sphaerocarpos donnellii* quantitative segregation might take place at both divisions. The cytological investigations bearing on chromosome reduction in insects were reviewed recently by Carothers (7). She concludes that, although either pre-reduction or post-reduction may occur, a given pair of chromosomes always behave in a predictable manner. Thus, one pair may always separate at the first division, another pair always at the second division, while a third pair may separate sometimes at the first division and sometimes at the second division.

	AB A11	Ab A12	ab A13	aB A14
AB A21	—	—	+	—
ab A22	+	+	—	—
AB A23	—	—	+	+
ab A24	—	+	—	—

TABLE 7.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae*. Cultures of the four sporidia from chlamydospore A1 (Minnesota) were paired with cultures from the four sporidia of chlamydospore A2 (Minnesota).

	AB 1	ab 2	Ab 3	aB 4
AB 1	—	+	—	—
ab 2	+	—	—	—
Ab 3	—	—	—	+
aB 4	—	—	+	—

TABLE 8.—Gall formation in corn plants inoculated with sporidia of *Sorosporium reilianum*. Cultures of the four sporidia from a single chlamydospore were used singly and in pairs. (The relative positions of the sporidia on the promycelium were not determined).

It has already been stated that the four sporidia from spore A1 were all different sexually, while those from spore A2 fell into two sexual groups only, numbers 1 and 3 being of one sex and numbers 2 and 4 of the other sex. As both chlamydospores were taken from the same smut collection, the question naturally arose as to the behavior of the eight monosporidial cultures when paired together. According to theory, positive reactions might be expected to occur only in the following combinations: A11 × A22, A11 × A24, A13 × A21, and A13 × A23. However, when the experiments were carried out, six fertile combinations appeared, only three of which would

have been expected on theoretical grounds. The results are shown in table 7. This suggests that in the sporidia of some chlamydospores more than two pairs of factors may be involved in determining sexual reaction.

Infection of Uninjured Plants

In the experiments already described, it was found that no galls were produced when individual monosporidial cultures of *U. zeae* were injected into young corn plants. The question still remained as to whether or not the germ tube from a single haploid sporidium is capable of penetrating the uninjured epidermis and causing infection. In view of Rawitscher's (29) conclusion that conjugation takes place in the tissues of the host plant immediately before spore formation, it would appear *a priori* that the haploid germ tubes are able to penetrate the epidermis and develop an extensive parasitic mycelium. However, Zillig (36) considered that the haploid sporidia of *U. violacea* were unable to cause infection, and Dickinson (14) has shown recently that no infection results when seedlings are inoculated with sporidia of one sex of *U. hordei* or *U. levis,* but only when sporidia of the two sexes are combined.

The ability of the haploid sporidia of *U. zeae* to cause infection of uninjured plants was tested by inoculating corn seedlings one week old with the following monosporidial cultures: (1) C31, (2) C33, (3) a mixture of C31 and C33. In earlier experiments these cultures had been found to be of opposite sex. One series of inoculations was made with a nutrient solution (1 per cent malt extract and 2 per cent dextrose) containing the sporidia which had grown in it, while a second series was made with sporidia grown in test tubes of potato-dextrose agar and then scraped off and suspended in sterile distilled water. The liquid containing the sporidia was drawn into sterile pipettes and dropped on the leaves of the young seedlings. After the inoculations were made, the plants were placed in a moist chamber for 48 hours, and then removed to the greenhouse bench. As a control, other plants were treated in the same manner with (1) fresh sterile nutrient solution and (2) nutrient solution from which the sporidia had been removed by passage through a porcelain filter.

Eight days after inoculation, the plants were examined for signs of infection. The presence of small chlorotic areas on the parts of the leaves where the sporidia had been placed was considered as evidence that penetration had taken place. Pieces of the epidermis were stripped off, stained in aniline blue, and examined under the microscope. In plants inoculated with two monosporidial cultures of opposite sex, the hyphae are quite large and can be made out even without the aid of any stain. In plants inoculated with a single monosporidial culture, however, the hyphae resemble very fine threads and it is often extremely difficult to find them.

TABLE 9.—*Inoculation of uninjured corn seedlings with single monosporidial cultures of Ustilago zeae and with two such cultures of opposite sex*

Inoculum	No. of plants inoculated	No. of plants infected
C 31, in nutrient solution	14	13
C 33, do	16	10
C 31 × C 33, do	14	13[a]
C 31, in distilled water	16	4
C 33, do	16	3
C 31 × C 33, do	16	6
Sterile nutrient solution	8	No injury
Filtrate from sterile nutrient solution	8	do

[a] Twenty-four days after inoculation, 9 of these plants were completely killed by galls which formed on the stems just above the soil level.

The results of the inoculation of uninjured seedlings are summarized in table 9. No difficulty was experienced in infecting uninjured seedlings with a single monosporidial culture. When the inoculations were made with sporidia suspended in the nutrient solution in which they had grown, the infective power of a single monosporidial culture was about equal to that of a mixture of two such cultures of opposite sex. But the plants inoculated with a single culture showed no signs of injury apart from the flecking of the leaves: whereas, 9 of the 14 plants inoculated with a mixture of two cultures of opposite sex were completely killed. A relatively light infection was obtained by inoculating with sporidia grown on nutrient agar and suspended in distilled water. In this series a greater number of infections was obtained with a combination of two cultures of opposite sex than with a single monosporidial culture. This apparent difference may be due, in part, to the difficulty of detecting a very light infection by a single monosporidial culture.

The greater number of infections obtained with sporidia suspended in nutrient solution as compared to those in distilled water might appear to be due to some substance in the solution which had an injurious action on the epidermal cells of the leaves, thus allowing the sporidial germ tubes to penetrate more readily. Yet, this could not have been the case, since the leaves treated with the filtrate from a solution in which sporidia had grown showed no sign of injury. It was noticed that the nutrient solution containing the sporidia spread evenly over the leaves and that the distilled water tended to collect in drops. The sporidia suspended in the nutrient solution, therefore, would come in closer contact with the epidermal cells and would be more likely to cause infection.

The Origin of the Diploid Mycelium

The sporidia of many of the smuts when grown on certain culture media conjugate in pairs. Maire (28) believed fusions to take place between the sporidia of *U. zeae,* and he published one figure illustrating this process. However, the nuclei were not shown, and his figure suggests the budding off of a new sporidium rather than the process of conjugation. Sartoris (31) also stated that the sporidia of *U. zeae* conjugate, but he gave no illustrations in support of his observations. Other workers have been unable to obtain any evidence that the sporidia of this species conjugate in pure culture. During the course of the investigations recorded in this paper, sporidia which were known to be of opposite sex were grown together on several different media but no sign of sporidial fusions was ever observed. Mixtures of the two kinds of sporidia were placed also on the surface of corn leaves floated on water in petri dishes, and after 48 hours they were removed and examined under the microscope, but the results of these trials were always negative. The question of sporidial fusions must, for the present, await further experiment, but it seems clear that the conditions which stimulate this process in many other smuts are without effect on the sporidia of *U. zeae.*

As a result of cytological investigations, Rawitscher (29) concluded that the pairing of nuclei and the formation of the diploid mycelium were brought about by the fusion of neighboring hyphae in the tissues of the host plant immediately before the formation of chlamydospores. Rawitscher's observations have been responsible for the view now generally accepted, *viz,* that the parasitic stage of *U. zeae* is passed almost entirely in the haploid condition, the diploid phase being restricted to a brief period immediately preceding spore formation. However, in a recent paper, Seyfert (32) stated that he was able to find mycelium possessing clamp connections and paired nuclei some distance from the point of gall formation.

On one occasion during the present investigation it was possible to observe the exact point at which fusion of the haploid mycelia and association of the nuclei took place. Sporidia from two monosporidial cultures of opposite sex were mixed together and dropped on the leaves of young corn seedlings growing in pots in the greenhouse. Immediately after inoculation, the plants were removed to a moist chamber for 48 hours, and then were placed on the greenhouse bench. Eight days after the inoculations were made, pieces of the epidermis were stripped from the leaves at the point of inoculation, stained in aniline blue, and examined under the microscope. In one preparation (Plate VII, 20) it was found that two sporidia lying close together had sent out germ tubes which penetrated the upper wall of the epidermal cell. The thread-like infection hyphae then grew across the cell and

came together to form a stout diploid hypha in which several pairs of nuclei could be seen. The contrast in the size of the haploid and diploid mycelia was very striking. At three points on the diploid mycelium there were protuberances suggesting clamp connections, but the cross walls could not be made out. In many other preparations, however, numerous clamp connections were found. One of them is shown in Plate VII, 21. The hypha bearing this clamp connection pushed its way out through the upper wall of the epidermis, grew along the surface of the leaf, and penetrated the epidermis again beside a stomatal cell.

The experiments described in the foregoing seem to show conclusively that the germ tubes from haploid sporidia are able to infect young corn plants. The mycelium resulting from these infections consists of very fine threads and apparently is incapable of further parasitic development. But, if a plant has been infected by two sporidia of opposite sex, the haploid hyphae may unite a short distance from their points of infection and produce a stout diploid mycelium bearing clamp connections. This actively parasitic mycelium develops abundantly about the point of infection and eventually produces the conspicuous smut galls.

In respect to their life histories, the most interesting point of difference between *U. zeae* and some of the other smuts is to be found, not in the duration of the diploid phase, but in the manner in which it is initiated. The germ tubes from the haploid sporidia of *U. levis* and *U. hordei* are unable to gain entrance to their respective host plants, and it is presumed that infection can be brought about only by diploid sporidia which arise by the conjugation of haploid sporidia of opposite sex. But it has never been shown conclusively that conjugation takes place between the sporidia of *U. zeae*; and, in view of the evidence which has just been presented, this process would appear to be unnecessary. Infection by this fungus is accomplished by germ tubes from the haploid sporidia, and the diploid condition arises almost immediately afterwards by fusion of two haploid hyphae of opposite sex.

Modification of Sexual Reaction by Mutation

It is now well known that prolonged growth on artificial media may bring about very important changes in monosporous cultures of certain heterothallic species. Attention was drawn to this phenomenon first by Kniep (23), who found mycelia with clamp connections and paired nuclei in two monosporous cultures of *Schizophyllum commune* which had been kept for about a year on artificial media. Blakeslee (4) found that (+) and (−) strains of Mucor, when kept in culture for some time, gradually lost their power of uniting with one another. A similar loss of sexual vigor was reported by Couch (10) in Dictyuchus and by Derx (12) in Penicillium.

Bauch (2) states that in young cultures of *Ustilago violacea* the (+) and (–) sporidia can be distinguished readily by their rate of growth on several nutrient substances but that, as the cultures become older, these differences tend to disappear.

It was shown by Christensen and Stakman (8) that cultures of *U. zeae* very frequently produce sectors which differ from their parent colonies in color, type of growth, and certain other characters. Monosporidial cultures of *U. zeae,* likewise, give rise to sectors of this kind. Some of these sectors exhibit very striking morphological variations from the parent cultures, and, although there is no ground for assuming that certain cultural characters are in any way correlated with a particular sexual reaction, the possibility of a sexual mutation during the process of variation is not excluded.

Up to the present, only two of the mutants have been compared with their parent culture in sexual reaction. These mutants appeared in culture A23 growing on potato-dextrose agar, and transfers made from them were grown separately in flasks containing nutrient solution. The resulting cultures of sporidia were mixed in pairs in all possible combinations with the parent culture and the three other monosporidial cultures—A21, A22, and A24—derived from the same chlamydospore. The mixtures of sporidia were then injected into young corn plants in the usual manner. The results

		AB A21	ab A22	AB A23	ab A24	AB A231	AB A232
AB	A21	—	+	—	+	—	—
ab	A22	+	—	+	—	—	—
AB	A23	—	+	—	+	—	—
ab	A24	+	—	+	—	+	+
AB	A231	—	—	—	+	—	—
AB	A232	—	—	—	+	—	—

TABLE 10.—Gall formation in corn plants inoculated with sporidia of *Ustilago zeae.* Cultures of the four sporidia from chlamydospore A2, and two mutants (A231 and A232) from one of these cultures (A23), were used singly and in pairs.

of the inoculations are given in table 10. Both of the mutants—A231 and A232—reacted like the parent with culture A24, but the parent reacted also with culture A22, whereas the two mutants gave a negative result when paired with this culture. It should be noted that the combination A23 ×

A22 produced only a few galls, and it is possible that, under certain conditions, the two mutants, when combined with culture A22, might also have induced the formation of small galls. However, the leaves of infected plants were examined for diploid mycelium bearing clamp connections but none was found. In view of these facts, the most probable interpretation of the results would seem to be that during the process of morphological mutation there occurred also some physiological change which rendered the two mutants incapable of uniting with culture A22.

Aerial Sporidia Produced from Infected Corn Leaves

Under certain conditions cultures of *U. zeae* produce long chains of aerial sporidia. This type of spore production was observed frequently in hanging-drop cultures. Figures of these sporidia have been given by Hitchcock and Norton (20), Clinton (9), and Stakman (33). During the course of the investigations herein recorded, some observations were made which suggest that these aerial sporidia may be produced in nature from infected corn leaves.

Portions of leaves heavily infected with the diploid mycelium of *U. zeae* were floated on distilled water in a petri dish. When, after about 48 hours, the leaves were examined under the microscope, it was found that branches from the mycelium in the epidermal cells had grown upward through the epidermis and produced chains of aerial sporidia. The branches appeared to push through the epidermis at the junctions of the cell walls. Occasionally, the branches bearing sporidia arose from mycelium in the epidermal hairs on the margin of the corn leaf. One of these hairs bearing branches with aerial sporidia is shown in Plate VII, 22.

There seems to be good reason for believing that these aerial sporidia are produced from the diploid mycelium within the host tissue. Stout hyphae, frequently bearing clamp connections, could be seen in the epidermal cells and several of the aerial branches were connected directly with these hyphae. Yet, the aerial sporidia borne on these branches appear to be haploid. When stained preparations were made from a culture of these sporidia, it was found that each cell contained but a single nucleus. Furthermore, six monosporidial cultures were made from them, but when they were injected separately into corn plants they failed to produce galls. It is possible that the aerial branch growing up from a diploid hypha contains a pair of conjugate nuclei and that, when sporidia are formed, some of them receive a nucleus of one sex and others a nucleus of the opposite sex. The manner in which these sporidia are formed requires further investigation.

The separation of two conjugate nuclei which have become associated in a single cell and the subsequent production of haploid sporidia are not

unknown in the smuts. Bauch (3) states that the diploid or fusion sporidia of *U. longissima* which are formed by the conjugation of two haploid sporidia of opposite sex may, in pure culture, produce once more haploid sporidia of the two kinds. Dickinson (13) found this process to occur in cultures of *U. hordei* and *U. levis*. Recently, Dodge (15) showed that the homothallic mycelium of the Ascomycete, *Neurospora tetrasperma*, may produce some unisexual conidia.

In view of the fact that aerial sporidia of *U. zeae* are produced from infected corn leaves under certain conditions in the laboratory, it is possible that this process occurs also in nature. During damp weather, moisture would collect on the infected areas of leaves and stems and there would be provided conditions suitable for the production of these sporidia. Later, when the moisture had evaporated, the sporidia would be carried off by the wind to other plants where they might cause new infections. Such a method of spore formation and dispersal would be important in disseminating the fungus in nature.

Formation of Anthocyan Pigments in Infected Leaves

When the leaves of Golden Bantam corn plants growing in the greenhouse are infected with the diploid mycelium of *U. zeae*, the cells about the infected area take on a bright red color. Apparently, this color formation is characteristic only of leaves infected by diploid mycelium, for it never was observed in leaves inoculated with sporidia of one sex. When portions of a leaf bearing these colored areas are examined under the microscope, it is seen that many of the epidermal cells contain a red coloring matter in solution in the cell sap. Occasionally this red substance separates out in crystals. By crushing a portion of the leaf in a mortar with a little distilled water and filtering off the resulting liquid, it is possible to obtain a clear red solution which changes to a bluish-green on the addition of a drop of alkali. The red color in the leaf apparently is due to an anthocyanin. When a little of the red solution is acidified with sulphuric acid and extracted with amyl alcohol the latter remains almost colorless, indicating that the pigment is in the anthocyanin (glucosidal) condition. By heating some of the acidified solution the anthocyanin is hydrolyzed, and the red material can then be extracted with amyl alcohol. The diploid mycelium evidently does not bring about hydrolysis of the anthocyanin in the infected leaves, but, through its action on the host cells, there results an accumulation of this pigment in the epidermal cells.

SOROSPORIUM REILIANUM

The experiments carried out with *S. reilianum* were of a preliminary nature, but the results obtained seem to be of sufficient interest to war-

rant a brief present mention. The chlamydospores used in the investigation were collected by Dr. H. P. Barss on corn in Oregon.

Spore germination

Fixed and stained preparations for a study of spore germination were obtained by the methods already described for *Ustilago zeae*. The various stages in the germination of the chlamydospores and the budding of the

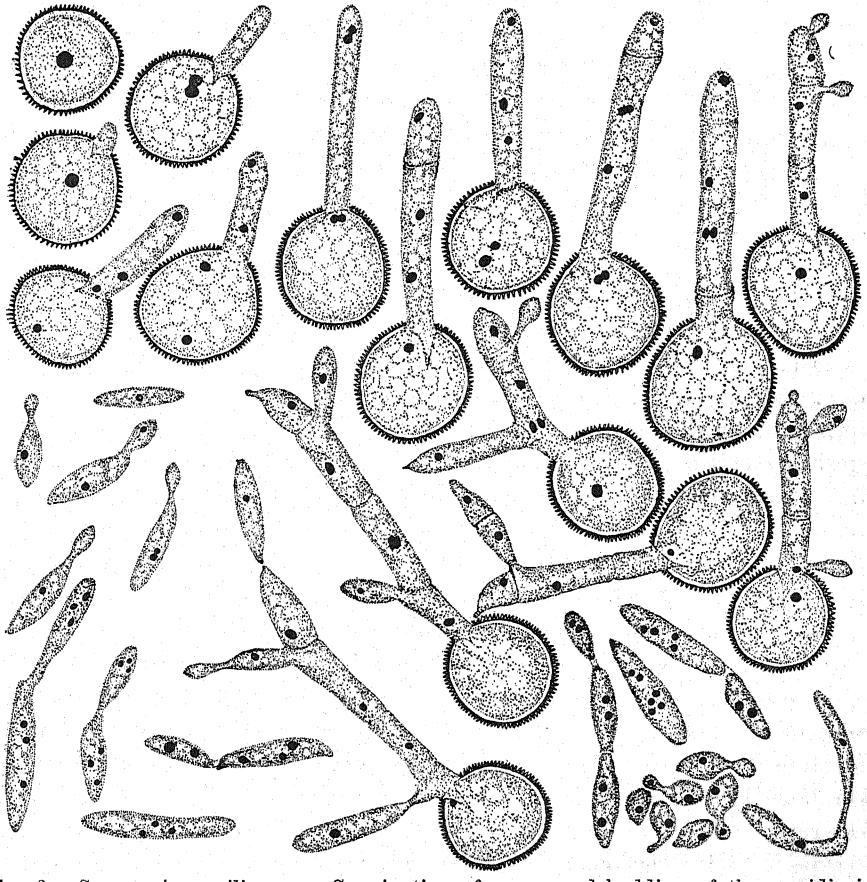


FIG. 3.—*Sorosporium reilianum*. Germination of spores and budding of the sporidia in culture. ×1330.

sporidia are shown in figure 3. The mature chlamydospore contains a single large nucleus. When germination begins, this nucleus divides, and subsequently each daughter nucleus undergoes a second division. Thus there are produced four nuclei, one of which remains in the spore, while the other three are distributed in the promycelium. Cross walls are then

laid down in the promycelium and sporidia are budded off from the promycelial cells. Meanwhile, the nucleus of each promycelial cell has divided and one of the daughter nuclei migrates into the sporidium, the other one remaining in the promycelial cell. In this way, the promycelium is able to produce more than one generation of sporidia. Many irregular forms of germination seem to occur, and frequently one or two promycelial cells may bear mature sporidia before other cells of the same promycelium begin to show any signs of sporidial production.

The sporidia borne on the promycelia and those produced from other sporidia by budding are generally uninucleate. Occasionally, however, the sporidia produced by budding may be binucleate or multinucleate. It is possible that the binucleate condition may arise by the conjugation of two sporidia of opposite sex, but, although sporidia have been found joined together in such a way as to suggest this process, in no instance did a nucleus appear to be in the act of migrating from one sporidium to the other.

Evidence of Heterothallism in Sorosporium reilianum

The four sporidia produced from a single chlamydospore were removed from the promycelium and cultured separately in flasks of nutrient solution. When a good growth of sporidia had been obtained, the monosporidial cultures were mixed in pairs in all possible combinations. Each monosporidial culture and each of the mixtures were then injected separately into young corn plants grown in the greenhouse. The results of the inoculations are given in table 8. The numbers 1 to 4 which have been given to the monosporidial cultures were chosen arbitrarily, since the relative positions of the sporidia on the promycelium were not ascertained.

Plants inoculated with combinations of cultures 1 and 2 and of cultures 3 and 4 produced galls bearing mature chlamydospores. All of the other combinations, and the monosporidial cultures alone, failed to produce galls. It is apparent, therefore, that *S. reilianum* is heterothallic and that a combination of two monosporidial cultures of opposite sex is necessary for the production of chlamydospores. Moreover, the sporidia used in this experiment must have belonged to four different sexual groups, since galls resulted in only two of the six possible combinations.

When corn plants growing in the field become infected with *S. reilianum*, the galls develop in the inflorescence. In this respect *S. reilanum* differs notably from *U. zeae*, for the latter may produce galls in almost any part of the plant. However, the young greenhouse plants infected with *S. reilianum* developed galls only in the leaves, and, although the plants were kept under observation until the time of flowering, no galls appeared in the inflorescences. Leaves bearing galls contained an abundance of stout hyphae

very similar in appearance to those found in leaves infected with the diploid mycelium of *U. zeae*. No hyphae could be found in leaves inoculated with monosporidial cultures of *S. reilianum*, or with those combinations of cultures which failed to produce galls. The red coloration, regularly present in leaves infected with the diploid mycelium of *U. zeae*, was observed also in the leaves bearing galls of *S. reilianum*.

When cultures of *S. reilianum* are grown on potato-dextrose agar, sectors appear frequently at the margins of the colonies. The possibility of a changes in sexual reaction during mutation has not been investigated.

Inoculation of Plants with Mixed Cultures of Ustilago zeae and Sorosporium reilianum

When a single plant is attacked by two species of smuts, or by two distinct races of the same species, opportunities for fusions between neighboring hyphae of the two kinds of mycelia must occur frequently. Fusions of this kind might then be followed by a pairing of nuclei from the two different kinds of mycelia, and this would result, at the time of spore formation, in the production of hybrid chlamydospores. Kniep (26) has shown that conjugation takes place readily between the sporidia of several different smooth-spore species of Ustilago, the (+) sporidia of one species fusing with the (–) sporidia of the other species. Conjugation was observed to take place also between sporidia of different echinulate-spore species, and between those of different reticulate-spore species. Likewise, conjugation occurred between sporidia of smooth-spore species and those of echinulate-spore species, but the pairing of sporidia from reticulate-spore species with those from species having smooth or echinulate spores gave only negative results. Dickinson (14) states that he was unable to infect oat seedlings with sporidia of the two sexes of *U. hordei* but, when sporidia of one sex of *U. hordei* were combined with those of the opposite sex of *U. levis*, infection resulted. The fusion of the two nuclei in the mature chlamydospores must be regarded as the final test of hybridization in the smuts. At the present time there is no record of the experimental production of hybrid chlamydospores.

U. zeae and *S. reilianum* sometimes occur on the same corn plant. As the two species are similar in many respects it seemed possible that under certain conditions they might hybridize. This possibility was tested experimentally by inoculating plants grown in the greenhouse with sporidia of the two species. The following cultures of *U. zeae* were used in the experiment: D11, D13, A11, A12, A13, and A14. These cultures were paired in all possible ways with the four cultures of *S. reilianum*, giving in all 24 mixed cultures. Each pair of cultures was then injected into eight corn

plants. The plants were examined frequently, but in none of them did any galls appear. It is probable, therefore, that hybridization does not take place between *U. zeae* and *S. reilianum*.

DISCUSSION

In considering the results of the preceding experiments it is of interest to compare briefly the relative importance of the parts played by the haploid and the diploid phases in the rôle of parasitism. Plants inoculated with single monosporidial cultures of *U. zeae* and *S. reilianum* apparently were not injured. In the case of *U. zeae*, which was more fully investigated, it was found that the haploid sporidia are able to penetrate the epidermal cells of uninjured corn leaves. But the delicate haploid hyphae seem to lack the vigor necessary for further parasitic development. It is only when two haploid hyphae of opposite sex unite to form a diploid mycelium that the pathogene becomes actively parasitic. Apparently the paired condition of the nuclei is associated with an increase in vigor which enables the invading hyphae to overcome the resistance of the host plant.

In the rusts, active parasitism occurs in both the haploid and the diploid phases. It is a curious fact, however, that, in the smuts, the parasitic habit is confined almost wholly to the diploid phase. The only apparent exception to this rule is to be found in *Doassansia sagittariae*, investigated by Rawitscher; and, in view of the more recent work by Seyfert, it is possible that in this species, too, infection is accomplished by means of haploid sporidia and that the diploid phase arises immediately afterwards by a fusion of two haploid hyphae of opposite sex. Whether or not this loss of parasitism in the haploid phase has been brought about by the habit of saprophytic development which the smuts have adopted would present an interesting subject for speculation.

In heterothallic species such as *U. zeae* and *S. reilianum*, parasitism is very closely linked with sexuality. The immediate result of the initiation of the sexual process is an increase in virulence; but the degree to which virulence is increased depends upon the particular mycelia of opposite sex which have been brought together. For example, a certain culture A, when combined with culture B of opposite sex, may produce relatively few galls but, when united with another culture C of the same sex as culture B, it may become extremely virulent. In the breeding of varieties resistant to these two smuts, therefore, it will be necessary to consider not only the plant's resistance to particular groups of physiologic forms but also its resistance to these forms when combined in various ways.

SUMMARY

1. The behavior of the nuclei during germination of the spores of *Ustilago zeae* and *Sorosporium reilianum* has been described and figured. In both

species the nuclei of the promycelial cells divide at the time the sporidia are formed and a daughter nucleus remains in each promycelial cell. In this way several generations of sporidia may be produced from a single promycelium.

2. Sporidial conjugations have not been observed. The primary sporidia of both species are uninucleate. The secondary sporidia of *U. zeae* produced by budding in culture are also uninucleate, but those of *S. reilianum* are occasionally binucleate or multinucleate.

3. Individual monosporidial cultures of *U. zeae* are able to infect young corn plants but they never produce galls. When a plant is inoculated with two monosporidial cultures of opposite sex, galls are formed which bear mature chlamydospores.

4. Some promycelia of *U. zeae* may bear two sporidia of one sex and two of the opposite sex. Sporidia of the same sex may be arranged in pairs on the promycelium or may alternate with those of the opposite sex. The promycelium of one chlamydospore produced sporidia belonging to four sexual groups.

5. Four monosporidial cultures from an Italian chlamydospore were paired with those from a Minnesota chlamydospore. The same two sexual groups were found to be present in both sets of cultures.

6. Sex in *U. zeae* appears to be determined by at least two pairs of factors. Segregation of these factors may occur at the first or the second division of the chlamydospore nucleus.

7. The haploid mycelium of *U. zeae* consists of fine hyphae and possesses weak parasitic powers. The diploid mycelium, which arises in the host plant by the conjugation of two haploid hyphae of opposite sex, is actively parasitic.

8. Mutants appear frequently in some monosporidial cultures of *U. zeae*. The morphological changes which occur during mutation may be accompanied by changes in sexual behavior.

9. When pieces of corn leaves infected with the diploid mycelium of *U. zeae* are floated on distilled water in a petri dish, hyphae grow out through the epidermis and produce chains of aerial sporidia. These sporidia appear to be haploid. The possible relation of these sporidia to the spread of the fungus in nature is discussed.

10. *Sorosporium reilianum* is heterothallic. Young corn plants inoculated with individual monosporidial cultures never produced galls, but plants inoculated with two monosporidial cultures of opposite sex developed galls containing mature chlamydospores.

11. Sporidia belonging to four sexual groups were produced from a chlamydospore of *S. reilianum*.

12. Mutants appeared in monosporidial cultures of *S. reilianum*.

13. Bright red areas occur in leaves of Golden Bantam sweet corn infected with the diploid mycelium of *U. zeae* or *S. reilianum*. This coloration, which is due to an accumulation of anthocyan pigments in the epidermal cells, was never found in leaves inoculated with a single monosporidial culture.

14. No galls were formed on plants inoculated with mixed cultures of *U. zeae* and *S. reilianum*.

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EXPLANATION OF PLATE VII

The development of *Ustilago zeae*.

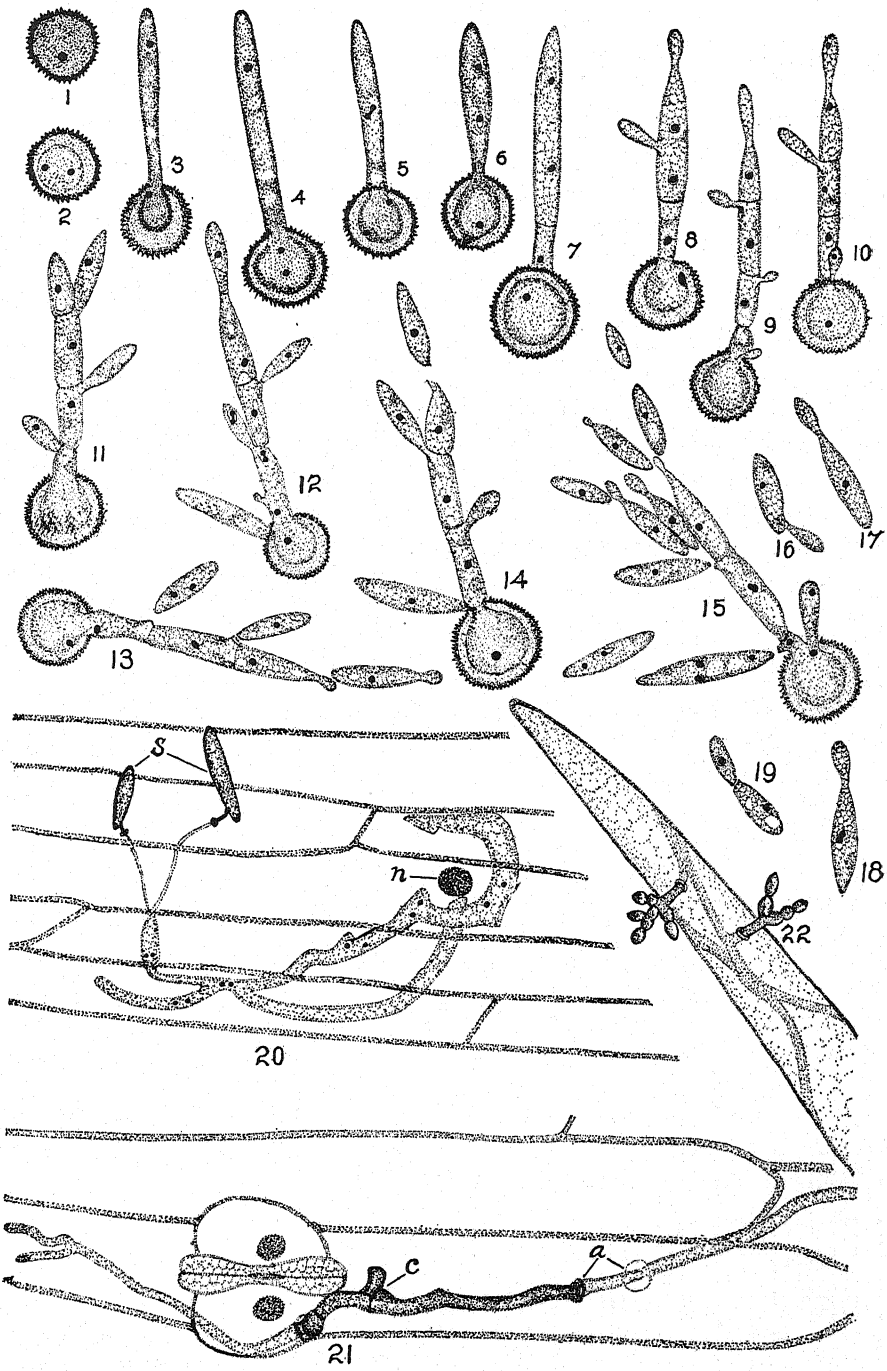
1–15. Spore germination. ×1330.

16–19. The budding of sporidia in culture media. ×1330.

20. Germ tubes from two haploid sporidia (S) of opposite sex penetrated the epidermal cell of a corn leaf and united to form the diploid mycelium. (N) Nucleus of epidermal cell. ×540.

21. Diploid hypha bearing clamp connection (C). The hypha came out through the epidermal cell of the leaf at (A), grew along the surface of the epidermis, and penetrated again beside the stomatal cell. ×540.

22. Mycelium in epidermal hair on margin of corn leaf bearing aerial sporidia. ×600.


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SPOROTRICHUM FRUIT SPOT AND SURFACE ROT OF APPLE[1]

MAX W. GARDNER[2]

HISTORY AND OCCURRENCE OF THE DISEASE

In Grimes, Winesap, and Ben Davis apples grown in southern Indiana, a shallow surface spotting or rot, caused by a fungus herein shown to be a strain of *Sporotrichum malorum* Kidd & Beaumont, was found on stored fruit (3).

The disease was first noted by C. E. Baker of the Department of Horticulture, in Grimes fruit taken out of air-cooled storage at the Purdue University orchard near Bedford, Indiana, in February, 1925. Mr. Baker found the disease again the next year in Grimes apples taken out of storage at the same orchard early in March (2, p. 234). In April, 1927, it was found causing heavy losses in a commercial crop of Winesap and Ben Davis apples grown near Seymour, Indiana, and stored in a local commercial cold-storage plant. At the time the writer visited the cold-storage plant, 200 bushels had been restored and 45 bushels had been discarded as worthless, due mainly to this disease, although there was also much secondary invasion of the lesions by blue mold. In 1928, Mr. Baker found the disease showing up as early as November 26 in Grimes apples in air-cooled storage at the Bedford orchard previously mentioned.

In the laboratory numerous isolations from the lesions were made each year and all yielded a slow-growing, grayish brown fungus with which successful inoculations have been made. This fungus proved to be a strain of *Sporotrichum malorum*, a culture of which was obtained for comparison. *S. malorum* was isolated by Kidd and Beaumont (4, p. 111) from rot lesions on apples in storage in England in the winters of 1921–1922 and 1922–1923 and was obtained in 30 out of the 1,795 of their isolations. The symptoms of the Delicious spot disease, a fungus storage rot described by Cunningham (1, p. 185) in New Zealand, rather closely resemble those of the disease under consideration, but no description of the causal fungus was given.

SYMPTOMS OF THE DISEASE

On the Grimes apples in air-cooled storage, the spots were 2 to 15 mm. in diameter, more or less circular with a rather indefinite margin, slightly sunken and light brown (amber brown or russet, according to Ridgway's

[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, La Fayette, Indiana.

[2] The writer wishes to acknowledge his indebtedness to Mr. C. E. Baker, of the Department of Horticulture, for his cooperation and to Professor H. S. Jackson for his advice and helpful criticism.

Color Standards) with a light tan (ochraceous-tawny) area at the center, apparently due to the drying of the rotted tissue under the skin at the center of the lesion (Pl. VIII, A, B, C; Pl. IX, B). Frequently, the lesions were numerous on a fruit and by their coalescence involved rather large areas. The lesions were shallow and the rotted tissue was badly disintegrated, shrunken, and either slimy or dried out and punky (Pl. IX, A).

The lesions on the other varieties were essentially similar except that they were somewhat deeper (Pl. IX, A) and, under the more humid conditions in the commercial cold storage, the rotted tissue did not dry out but remained soft and watery (Pl. VIII, D, E). This rotted tissue, together with the skin, sloughed off very easily when the fruit was handled, exposing the sound white tissue not far beneath the surface, and badly affected fruits slipped out of one's hand very easily. On the Ben Davis fruits there was frequently a transverse crack in the skin over the lesion. When affected fruits were held in a damp chamber, there was a scanty development of gray aerial mycelium.

Kidd and Beaumont (4, p. 111), in their brief description, state that the lesions "are dark brown and rather dry and arise at a lenticel spot, insect wound, or at the calyx." The relation to the calyx or insect wounds was not observed on the Indiana apples but many of the lesions were centered about lenticels. Forty-seven lesions on Grimes were examined with this point in mind and 30 were found to be centered about lenticels. On Ben Davis about half of the lesions were so situated but, on Winesap, only 16 out of 101 lesions examined were centered about lenticels.

Ordinarily the lesions were not over 1 or 2 mm. deep although, on the apples in the commercial cold storage, they were about 5 mm. deep (Pl. VIII, E) and, in certain inoculation tests, even deeper lesions occurred (Pl. IX, F, G). The brown rotted tissue was spongy or loose and stringy, much shrunken, sharply delimited, and easily removed from the sound underlying tissue. Microscopic examination of water mounts of the rotted tissue and of razor sections through the lesions showed that the large parenchyma cells under the subepidermal collenchyma were completely collapsed and their walls brown and crumpled but more or less intact. There was an abundance of fine, sparsely branched, colorless mycelium and cylindrical, colorless, one-celled spores between and within the large parenchyma cells (Pl. X, G, H). The epidermal cells and the thick-walled subepidermal cells usually were not collapsed. The collapsed parenchyma cells were readily separated in the water mount and freely floated about, each containing the mycelium and spores of the fungus (Pl. X, G, H) and frequently few or none of the starch grains so conspicuous in the uninvaded cells. The great abundance of the characteristic mycelium and spores in

the rotted tissue, within as well as between the cells, serves as an excellent diagnostic character.

ISOLATION OF THE FUNGUS

Tissue plantings in potato-agar plates were made from 20 lesions on the Grimes apples brought in by Mr. Baker, in 1925, and all yielded apparently pure cultures of the same fungus (Pl. IX, H). Similar plantings were made from 70 lesions on the Grimes fruit in 1926 and all but one yielded the same fungus isolated the year before. Plates were poured from a suspension of the spores in certain of these lesions and were thickly seeded with the same fungus.

Tissue plantings were made from 113 lesions on 16 Ben Davis fruits from the commercial cold storage in 1927, and 75 yielded the same fungus previously isolated, 41 in apparently pure culture. Plantings were likewise made from 58 lesions on four Winesap apples and the same fungus was obtained from 56 and in apparently pure culture from 54. The fungus was isolated also from Grimes apples of the 1928 crop. Furthermore, the fungus has been repeatedly reisolated from apples inoculated in the laboratory.

MORPHOLOGY OF THE FUNGUS

The hyphae are of comparatively small diameter, the young colorless ones averaging about 2 μ in diameter, the older brownish ones about 3 to 4 μ. The hyphae are sparsely branched and form long unbranched strands; the presence of numerous globules gives the contents a beaded appearance. Simple circular loops or coils about 10 to 12 μ across and also coarse, brown, composite strands of about 3 to 10 or more parallel adherent hyphae are produced in the aerial mycelium in culture and in the subepidermal cavities in the lesions (Pl. X, F). The composite strands apparently are the result of the tendency of the successive lateral branches of the parent hyphae to remain adherent and parallel to, but not intertwined with, the parent hyphae. In the condensation moisture about the margin of a hanging-drop culture, the long unbranched hyphae sometimes produce large flat compound coils 20 to 30 μ across (Pl. X, E). Rounded swollen, brown, chlamydospores are found in old cultures.

The conidiophores are short lateral ten-pin-shape hyphae typically about 5 to 8 μ in length and swollen at the center and toward the base. Apparently, the conidiophores may continue to elongate after they begin to produce spores and frequently attain a length of 20 μ (Pl. X, A, B, C). In hanging-drop cultures in prune decoction the conidiophores arise at more or less of a right angle and are usually solitary. (Pl. X, A, C). In the aerial mycelium, however, they arise at a more acute angle and, in the badly rotted host tissues, are frequently in terminal or lateral clusters

or groups, (Pl. X, D), suggestive of Verticillium except that the conidiophores are apparently not in definite whorls. The conidiophores usually are not opposite on the main hypha (Pl. X, B, C) but in the denser clusters are, of course, much more nearly so. In these clusters the lower portion of each conidiophore is often rather closely appressed to the main hypha while the apical portion curves outward.

The spores are colorless, cylindrical or ellipsoidal, one-celled, 4.6 to 8.4 μ in length and 1.4 to 2.1 μ in breadth. The spores are abstricted one after the other from the apex of the conidiophore, each being pushed off by its successor (Pl. X, A, B, C), and in aerial mycelium, or mycelium submerged in agar, accumulate about the tip of the conidiophore in a packet or clump.

CULTURAL CHARACTERS OF THE FUNGUS

The fungus grows readily but rather slowly on the ordinary culture media. In potato-dextrose agar, colonies from spores are white, prostrate, and about 1 mm. in diameter in two days and 9 mm. in 7 days, at about 22° C., with pale, olive buff (Ridgway's Color Standards), cottony, aerial mycelium, on an olive brown to dark olive central area in the substrate and a white border of prostrate radiating hyphae (Pl. IX, K). On older colonies, the aerial mycelium becomes drab gray to smoke gray and the prostrate border may be dark colored (Pl. IX, H). On agar with a lower water content, the aerial mycelium is more velvety and the prostrate marginal zone much narrower. Slant cultures on potato-dextrose agar are soon covered with the grayish aerial mycelium on an olivaceous substrate.

On cornmeal agar, the fungus is prostrate with radiating hyphae and white with the olive brown color appearing at the center much later than on potato dextrose agar and much less pronounced. Cornmeal-agar plates containing large colonies were flooded with an alcohol solution of iodine and evidence of the disappearance of starch under the colonies was obtained. On water agar, containing soluble starch, this test gave more striking evidence that the starch under the colonies had been utilized.

When the fungus was grown on slants of nutrient agar, containing dextrose as a carbon source, and the hydrogen-ion indicators brom thymol blue or brom cresol purple, acid production was indicated by the change to a yellow color. After about 10 days, however, the alkaline color reappeared.

In a test of the effect of temperature on the growth of the fungus on potato dextrose agar, it was found that the average increase in colony diameter in one week was 9 mm. at 12° C., 15 mm. at 17°, 19 mm. at 19°, 22 mm. at 23°, 23 mm. at 27°, 15 mm. at 30°, and 1 mm. at 34°. The maximum radial growth rate is about 1.5 mm. per day. Spores were

produced at all temperatures, and chlamydospores were produced at 30° and 34°. The optimum temperature for growth seems to be between 23° and 27°, but the fungus grows well at lower temperatures and is able therefore to grow slowly in the apples in storage.

The spores germinate promptly in water, prune decoction, and other liquid media, and, on the mycelium thus produced, sporulation is abundant within 3 days. In sterile tap water, germination occurred readily at 12°, 17°, 19°, 23°, 27° and 30° C., and in buffer solutions at hydrogen-ion concentrations from P_H 8.0 to P_H 3.4. Germination was most vigorous at P_H 5.8 to P_H 4.4.

PATHOGENICITY OF THE FUNGUS

The pathogenicity of the fungus was proved by placing slabs from agar plate cultures on the unbroken skin of the apple and also over small scalpel incisions. Inoculations were also made by inserting mycelium or spores into scalpel incisions. On apples thus inoculated and kept 1 to 4 weeks in damp chambers at room temperatures, lesions were found about the incisions, and the presence of the typical mycelium and spores in the invaded tissues together with successful reisolations confirmed the identity of the fungus.

Invasion through the unbroken skin under agar slabs was obtained on fruit of the Grimes, Jonathan, Winesap, Stayman, Rome, Wealthy, Ben Davis, and Gano varieties. The lesions occurred only in the areas covered by the slabs (Pl. IX, C, E) and were usually not over 1 mm. in diameter when the records were taken 1 to 4 weeks later. Many, but not all, were centered around lenticels (Pl. IX, D). Control fruits on which plain agar slabs were placed developed no lesions. Among a total of 31 fruits inoculated, 27 developed these small lesions under the agar slabs. Tissue plantings in agar plates were made from 87 of these lesions and the fungus was cultured from 63. Razor sections through lesions under agar slabs on Grimes fruit, after an incubation period of 10 days, showed the mycelium in the large parenchyma cells of the 13th and 14th cell layers.

Attempts to infect apparently uninjured fruit with a spore suspension prior to storage have been successful and it was found that a very long time was required for the formation of the rather large lesions in storage. On October 12, 1927, 24 Grimes and 12 Stayman fruits were dipped in a spore suspension, held in a damp chamber 24 hours, and then held in paper sacks in cool storage at 15° C. On November 8, no infection was found and the fruit was transferred to a cooler storage room (2° C). On December 15 no lesions were found, but on February 8, one lesion, 12 mm. in diameter and 10 mm. deep, was found on one fruit. There was a cavity under the skin of the lesion due to the shrinkage of the dry spongy or fluffy rotted tissue. This tissue contained an abundance of the spores and the fungus

was reisolated. On March 9, a more or less similar lesion (Pl. IX, F. G) was found on each of six more fruits, from five of which the fungus was reisolated. Twelve fruits of each variety dipped in water instead of the spore suspension and stored under similar conditions to serve as controls remained free from infection.

An additional 12 Grimes fruits, dipped in a spore suspension at the same time, and incubated 24 hours in moist chambers, were stored in sacks in a refrigerator at about 12° C. On February 1, two lesions were found on one fruit and one on a second, ranging from 6 to 10 mm. in diameter and containing the typical spores of the fungus. The fungus was reisolated from each fruit by means of plates poured from a suspension of the spores from the rotted tissues. Twelve fruits dipped in water instead of the spore suspension to serve as checks developed no lesions.

The length of time necessary for the formation of these lesions and the very small size of the lesions in the agar-block inoculations indicate that the lesions found in storage probably develop very slowly. Lesions have never been seen in the orchard nor on recently harvested apples. The Grimes apples on which Mr. Baker found the disease had been carefully inspected by him before they were placed in storage and were found free from all blemishes. Large lesions have been found only after a rather long period of storage. The agar-slab inoculations, however, show that the fungus can infect recently harvested apples, since infection was obtained in September and October as well as later in the storage season. Attempts to infect small green fruits in May were unsuccessful. Just when natural infection occurs and what are the sources of natural infection are not known.

CAUSAL FUNGUS A STRAIN OF SPOROTRICHUM MALORUM

The mode of spore formation indicated that the fungus was probably a species of Sporotrichum, although it showed certain morphological characters in common with Verticillium and Cephalosporium. Cultures of the maple-wilt Verticillium and *Cephalosporium acremonium* were available for comparison. Saccardo (7, p. 870) lists a number of species of these genera on apple wood or fruit, all of which differ in some respect from the fungus under consideration. Among the species described on fruit, *Verticillium lateritium* Berk. differs in color of mycelium and shape of spores (6, p. 156), *V. epimyces* B. & Br. var. *epicarpium* Sacc. in color and shape of spores (6, p. 154), and *Sporotrichum Thuemenii* Sacc. in shape of spores (6, p. 104). In addition to these, Oudemans (5, p. 496), in his list of species on the fruit, includes *Sporotrichum lyococcon* Ehrb., which also differs in shape of spores (6, p. 99).

A culture of *Sporotrichum malorum,* recently described by Kidd and Beaumont (4, p. 111) as the cause of a rot of apples in England, was obtained from the Centraalbureau voor Schimmelcultures at Baarn, Netherlands, and was found to be similar to the Indiana fungus. The two fungi were carefully compared as to morphology, cultural characters, and pathogenicity and, although certain differences were found, these were considered insufficient to warrant the necessity of placing the Indiana fungus in another species.

The two fungi were essentially similar in mycelial characters and in size, shape, and mode of production of the spores. The spores of the Indiana strain were slightly broader and were produced more abundantly in culture. The British strain grew slightly more rapidly in culture, the colonies on the 12th day exceeding in diameter those of the Indiana strain by about 4 mm. There were slight differences in pigmentation. In terms of Ridgway's Color Standards the aerial mycelium of the Indiana strain on potato-dextrose agar was smoke gray and the substrate or under side of the colony was olivaceous black, while the aerial mycelium of the British strain was pale smoke gray and the substrate, dull greenish black. On cornmeal agar, the Indiana strain was darker than the British strain (Pl. IX, J), but the difference was not always so marked as in the colonies illustrated. The two strains were similar in acid production and starch utilization.

Inoculation tests with the British strain were made along with some of those previously described and very similar results were obtained. The lesions produced were similar to those obtained with the Indiana strain both as to effect on the host tissue and the presence of mycelium and spores between and within the cells. Small lesions were formed under slabs from agar-plate cultures placed on the unbroken skin on Grimes, Rome, and Ben Davis fruits (Pl. IX, C) and the fungus was reisolated from 74 out of the 116 lesions tested. The spores were not as numerous in the tissues as in the similar lesions obtained with the Indiana strain.

Out of 12 Grimes fruits, dipped in a spore suspension from the British strain on October 12, 1927, held in moist chambers 24 hours, and then stored in sacks in a refrigerator, five had developed by March 2 one or two rather large lesions, in some instances apparently about wounds. The rotted tissue in the lesions was brownish or dark gray and dry and spongy and contained the typical mycelium and spores. Reisolations were made by the poured-plate method from the spores in six of these lesions, representing each of the infected apples, and practically pure cultures of the fungus were obtained.

Therefore the culture of *Sporotrichum malorum* closely resembled the Indiana fungus in pathogenicity as well as in morphology, and the slight differences in culture are not considered of specific significance. Conse-

quently, it seems justifiable to consider the Indiana fungus as a strain of *Sporotrichum malorum*.

SUMMARY

In Grimes, Winesap, and Ben Davis apples grown in southern Indiana, a shallow surface spotting of fungus origin was found when the fruit was removed from storage.

The lesions were 2 to 15 mm. in diameter, more or less circular with a rather indefinite margin, slightly sunken, and light brown with a light tan area at the center. The lesions were shallow and the rotted tissue, either shrunken and dried out or soft and watery, was easily scraped from the sound white underlying tissue.

The rotted tissues contained an abundance of the mycelium and spores of the causal fungus not only between but also within the large parenchyma cells. These cells were collapsed and separated and many had lost their starch content.

The causal fungus was readily isolated. The hyphae are sparsely branched and frequently form loops or simple coils. In the aerial mycelium compound strands of parallel hyphae are produced. Cylindrical, unicellular, colorless spores are abstricted, one after another, from the tips of unbranched, ten-pin-shape conidiophores.

In the rotted tissues the conidiophores are frequently in terminal or lateral clusters, somewhat suggestive of Verticillium, but the conidiophores are apparently not in definite whorls.

The colonies of the fungus on potato agar have a prostrate margin of radiating hyphae and at the center fluffy, buff or gray, aerial mycelium on an olivaceous substratum. The colony diameter increases at the rate of about 3 mm. per day. The fungus temporarily increases the acidity in dextrose agar and utilizes starch. Best growth occurs between 19° and 27° C. and good growth occurs even at 12° C. The spores germinate readily in a wide range of temperatures and hydrogen-ion concentrations.

The pathogenicity of the fungus was proved by placing slabs from agar plate cultures on the unbroken skin of the fruit and by dipping apples in a spore suspension prior to storage. Infection was obtained on Grimes, Jonathan, Winesap, Stayman, Rome, Wealthy, Ben Davis, and Gano fruits. Infection occurred apparently through the unbroken skin, through lenticels, and through wounds. The fungus was reisolated.

A very long incubation period was necessary for the formation of large lesions. Although the fungus can invade freshly harvested apples, only very small lesions were produced, and large lesions have been found only on apples that have been stored for a rather long period.

A culture of *Sporotrichum malorum,* isolated from apples in England by Kidd and Beaumont, was obtained for comparison and in morphology, cultural characters, and pathogenicity to apples proved to be so like the Indiana fungus that the latter is considered a strain of that species. The British strain differed mainly in its slightly faster growth, lighter pigmentation on cornmeal agar, and less profuse sporulation in culture. The lesions which it produced on apples were very similar to those produced by the Indiana strain as was also its effect on the host tissues.

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EXPLANATION OF PLATES

PLATE VIII

A. Sporotrichum lesions on Grimes apple taken out of air-cooled storage early in March, 1926.

B and C. Sporotrichum lesions on Grimes apples taken out of air-cooled storage in February, 1925.

D. Advanced stages of Sporotrichum surface rot on Ben Davis apple taken from commercial cold storage in April, 1927. Rotted tissues, soft and watery.

E. Cross section of a Ben Davis apple in commercial cold storage showing shallow penetration of the surface lesions.

PLATE IX

A. Sporotrichum lesions on Winesap apple in commercial cold storage, April, 1927. Skin shaved off to show appearance of rotted tissues.

B. Small Sporotrichum lesions on Grimes apple from air-cooled storage, 1926, enlarged to show the light center and indefinite margin.

C. Lesions on Grimes apple inoculated by placing a slab from an agar culture of the British strain on the unbroken skin. ×2.

D. Lenticel lesions on Stayman apple inoculated 12 days previously (February 1, 1926) by placing slab from agar culture of the Indiana strain on the unbroken skin. ×6.

E. Lenticel lesions on Grimes apple inoculated 12 days previously (February 1, 1926) by placing slab from agar culture of the Indiana strain on the unbroken skin. ×2.

F and G. Sections through large deep lesions on Grimes fruits inoculated by dipping in a spore suspension in October and held in cold storage until March.

H. Sporotrichum colonies from tissue plantings in potato-dextrose agar. Isolations from lesions on Grimes apples, February, 1925.

J. Difference in rate of growth and pigmentation of 13-day-old colonies of the Indiana (above) and British strains on cornmeal agar. The British strain produces less pigment on this medium and grows slightly faster.

K. Six-day-old colonies from spores in a poured plate of potato-dextrose agar.

PLATE X

A to C. Conidiophores and conidia in condensation moisture about the margin of hanging-drop cultures in prune decoction. Indiana strain. Cultures 5 days old. ×850.

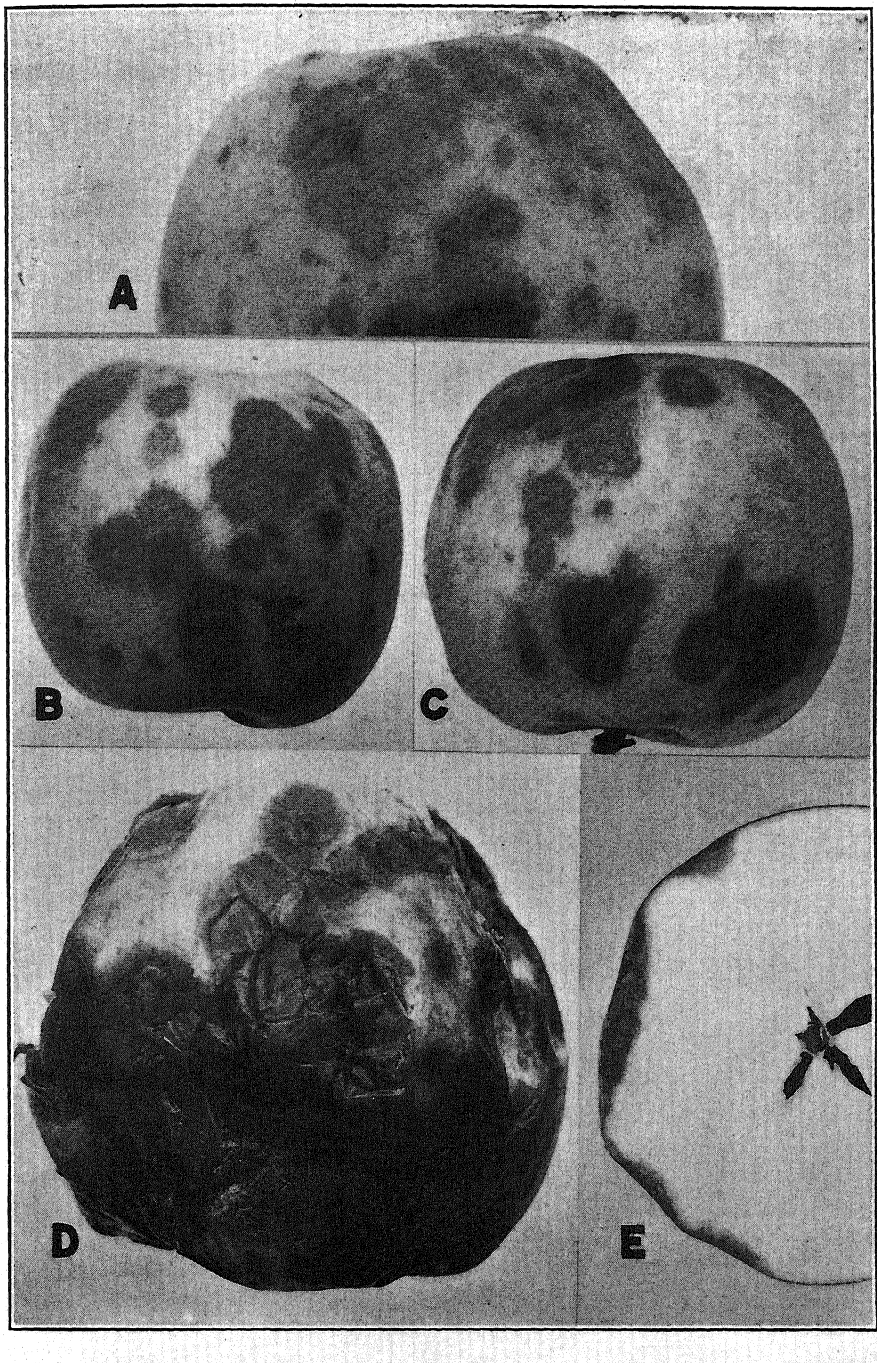
D. Conidiophore clusters produced in rotted tissue. The central cluster is from an apple inoculated with the British strain as described below under G. (×600.) The other two clusters are from naturally infected apples. (×750.)

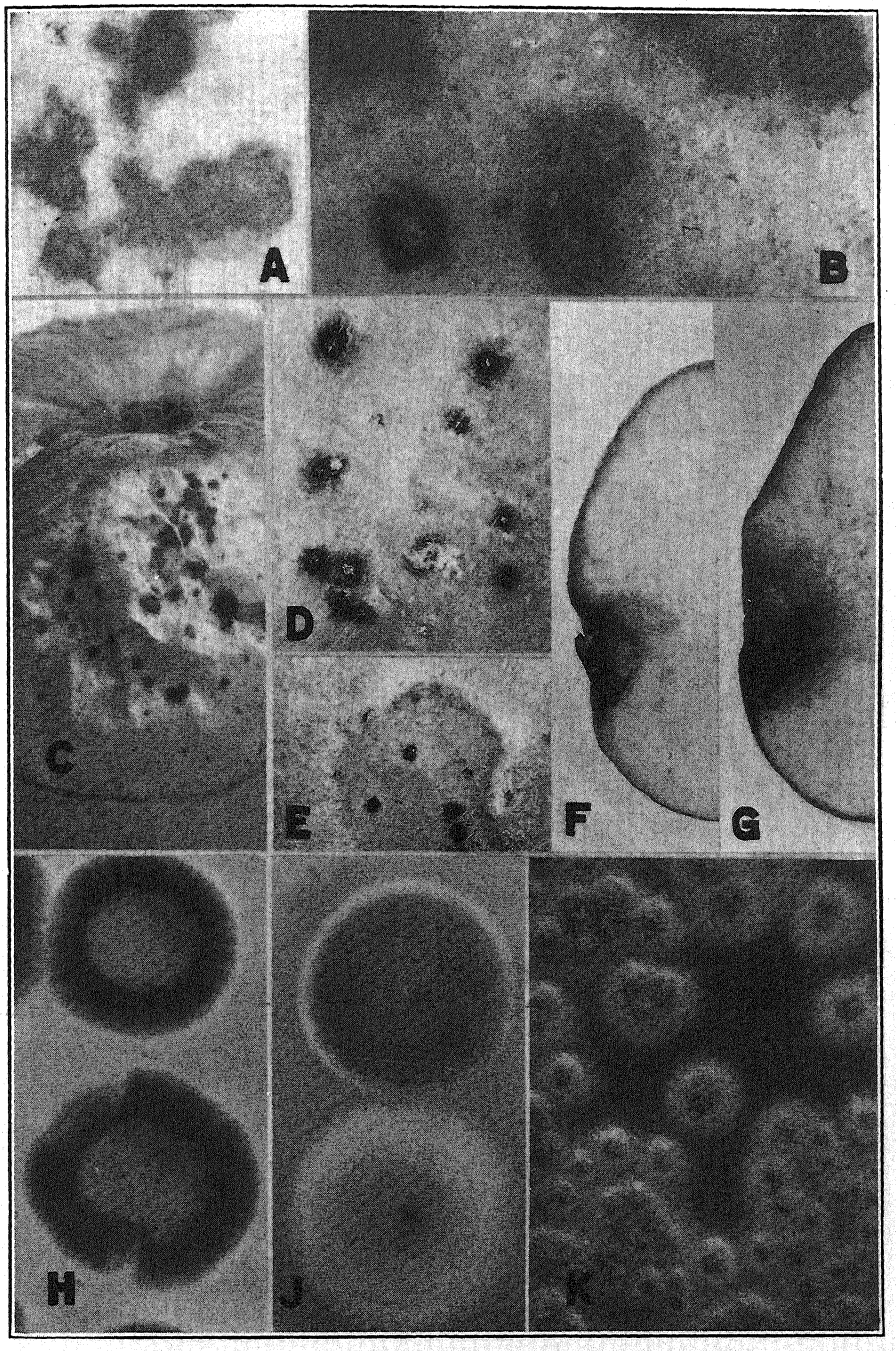
E. Compound hyphal coil in condensation moisture about the margin of hanging-drop cultures in prune decoction, 6 days old. Indiana strain. ×850.

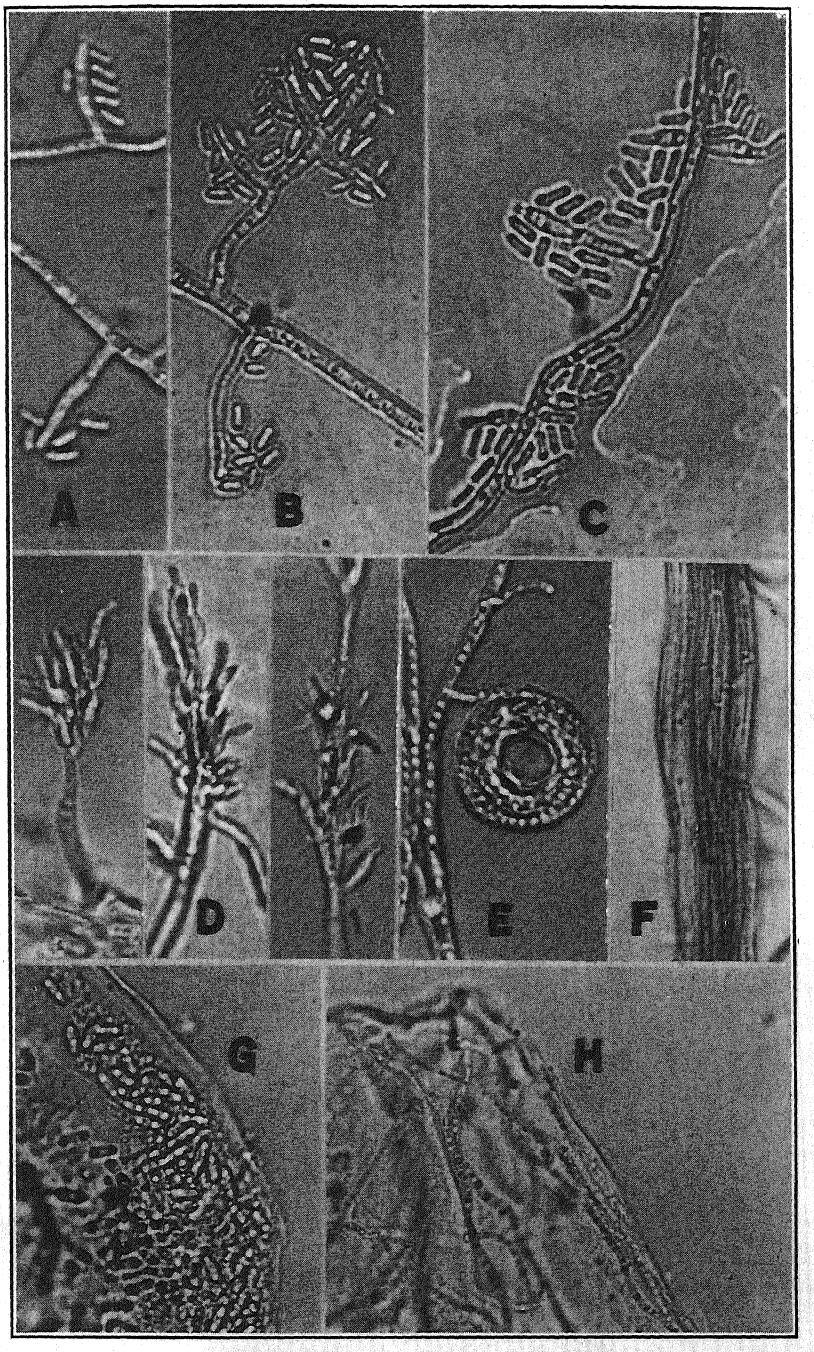
F. Composite strand consisting of numerous parallel hyphae. From aerial mycelium on culture of Indiana strain. (×750.)

G. Portion of a parenchyma cell in a water mount of rotted tissue from a lesion on a Grimes apple showing the numerous spores within the cell. The fruit was inoculated in October by dipping it in a spore suspension from the British strain and removed from storage in March. ×675.

H. Portion of a parenchyma cell in a water mount of rotted tissue from a lesion on a Grimes apple showing the mycelium within the cell. Fruit inoculated as noted above in G. ×425.


A
B
C
D
E


A
B
C
D
E
F
G
H
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K


A
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CRONARTIUM COMPTONIAE ARTH. IN WESTERN NORTH AMERICA

H. G. LACHMUND

INTRODUCTION

Cronartium comptoniae Arth., a serious blister rust of pitch and hard pines, alternating to members of the family Myricaceae, has been known in North America since 1906, when it was named by Arthur (1). Connection of the uredinial and aecial stages was established by Clinton in 1907. Subsequent work by various investigators (4 to 10, 12, 13, and 15 to 20) showed the disease to be indigenous, ranging from New Hampshire south to North Carolina and west to Minnesota and Missouri. The disease is characterized as very harmful to young pines both in the nursery and in the forest and especially destructive to introduced western pines (15). The need for keeping it out of the Pacific Northwest where the range of the alternate host, sweet gale (*Myrica gale* L.), was known to overlap portions of the commercial ranges of western yellow pine (*Pinus ponderosa* Laws.) and lodgepole pine (*P. contorta* Loudon) was emphatically pointed out by Rhoads and others (15) in 1917. The importance of these species, particularly of western yellow pine, in this section, together with the evidence of their susceptibility developed in the East, fully justified this warning. Botanical exploration of the Pacific Northwest had not then been sufficiently intensive to provide details on the extent of association of sweet gale with pines. The purpose of this paper is to report the discovery of this disease and the results of subsequent study of it in this territory. The rust is indigenous here as well as in the East.

WESTERN DISCOVERY, DISTRIBUTION, AND HOSTS

Credit for the discovery goes to Mr. H. N. Putnam of the Office of Blister-Rust Control and to Dr. J. S. Boyce of the Office of Forest Pathology, who first noticed the disease on sweet gale (*Myrica gale*) at Daisy Lake, British Columbia, on October 13, 1924. Telial material was collected and sent for determination to Spaulding who identified it tentatively as *Cronartium comptoniae*. On May 5, 1925, the writer collected aecial material showing the characteristic tooth-like margins along the dehiscence of the peridia from *Pinus contorta* in the vicinity of the sweet gale at Daisy Lake. Part of this material was sent for further examination and use in cross inoculations to Spaulding who, with suitable precautions, successfully infected *Myrica asplenifolia* L. (*Comptonia peregrina* L. Coulter) with it, and definitely established its identity as *Cronartium comptoniae*. Since discovery of the rust at Daisy Lake, knowledge of its western distribution has

been extended to include records of it from a number of widely separated localities, the northernmost of which to date has been Prince Rupert, British Columbia, and the southernmost located at Ilwaco, Washington, at the mouth of the Columbia River. Infection was found on pine and sweet gale at both of these limits.

In the East, the hosts listed (15) for the aecial and pycnial stages are the following species of Pinus: *banksiana, contorta, densiflora, echinata, jeffreyi, maritima, montana, mugho, nigra austriaca, ponderosa, pungens, resinosa, rigida, sylvestris, taeda,* and *virginiana;* and for the uredinial and telial stages: *Myrica asplenifolia* (*Comptonia peregrina*), *M. gale,* and *M. carolinensis.* In the West, the rust has been found on lodgepole pine and sweet gale, only. No associations of sweet gale with other susceptible pine species have yet been recorded. Within the range of the rust here, sweet gale is a plant of very local occurrence, limited to certain types of swampy ground in bogs and along lake margins. South of British Columbia, it is uncommon. The vegetation with which it commonly is associated and its habitat render it both inconspicuous and inaccessible. The aecial host, lodgepole pine, is inferior among Pacific Coast timber trees. Its diseases have received relatively little attention. Furthermore, detection of the disease on it is complicated by the similarity in appearance of another closely related, much more common and widely distributed, native rust of the same host, *Cronartium filamentosum* (Peck) Hedgc. & Long. These conditions, added to the fact that the Pacific Northwest is comparatively new territory in mycological exploration, readily explain why the disease was not discovered earlier.

The complete distribution of the disease as now known is shown in figure 1. Localities in which it has been found on the uredinial and aecial hosts are indicated by dots and triangles, respectively. The altitudinal range of these localities in British Columbia, for both pine and sweet-gale infection, extends from sea level to 1400 feet. Ilwaco, Washington, the only recorded locality of occurrence south of the International Boundary, is at sea level.

CHARACTER OF INFECTION AND DAMAGE

On sweet gale, the rust causes a characteristic spotting of the leaves. The yellowish uredinia on the dorsal surface are soon followed by telia. The brownish telial spore horns develop in dense, more or less matted, tufts and are produced in abundance. Even with quite heavy infection, little injury is evident on this host.

Infection of the pines, as in the East (18, 13), takes place only on the youngest seedlings. Apparently, it occurs very sparingly, if at all, on trees

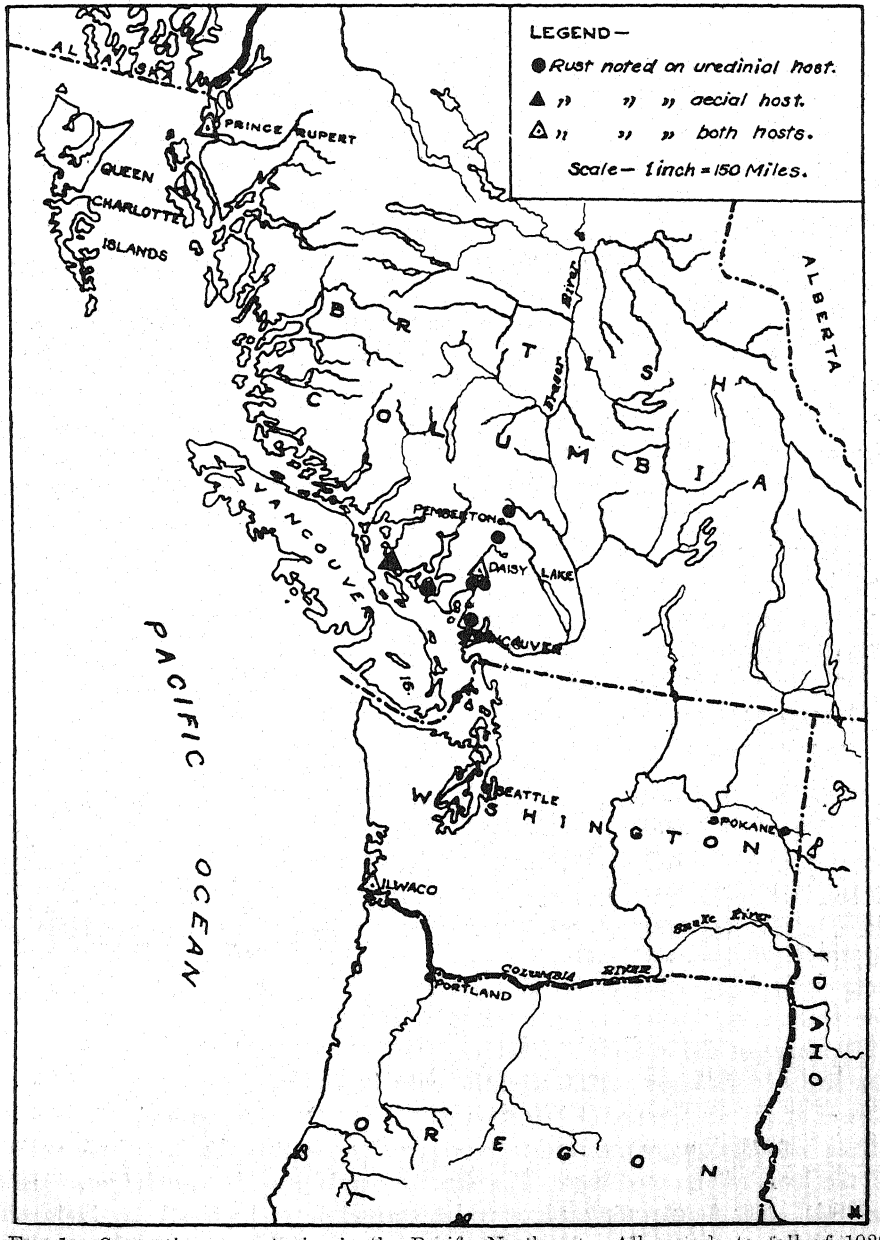


FIG. 1. *Cronartium comptoniae* in the Pacific Northwest. All records to fall of 1928.

more than 10 or 12 years of age and only the basal portions of the tree are susceptible. The incipient cankers seem to make their appearance mainly in the first or second season following infection and rapidly develop into long, fusiform swellings. Since the cankers are almost invariably on branches or stems near the base of the tree, the swelling soon spreads to ground level and the infection usually appears to start there and extend upward into the lower branches. In the earlier stages, canker growth proceeds vigorously. The swelling increases and spreads rapidly at a rate of 2 to 6 inches a year. Aecia are produced in profusion from the swollen bark. Growth of the seedling generally is greatly retarded and the tree becomes badly stunted or deformed. This, together with the formation of pathological adventitious shoots, often gives the whole seedling the character of a short witches' broom. During the susceptible, juvenile period, a considerable proportion of the trees may be killed.

At Daisy Lake, British Columbia, a patch of about 500 young trees of *Pinus contorta,* ranging from 4 to 12 years of age, occurs on a knoll on a small point of land extending into Lake Lucile. Numerous plants of sweet gale are distributed along the shore on two sides of the point. The distances separating the pines from the sweet gale range from 15 to 50 feet and the pines are heavily infected. A sample of 134 trees examined indiscriminately through the patch on November 1, 1925, may be considered representative of the entire patch.[1] The results of the examination of these trees are given in tables 1 and 2. The character and extent of the infection here is shown in table 1.

Table 1 shows that the great majority of the trees (127 out of 134, or approximately 95 per cent) are from 5 to 10 years old, with most of them between 6 and 8 years. Eighty-nine, or about 65 per cent, are infected. A total of 94 cankers was found on these trees. The confinement of infection to the basal portions of the tree is clearly evident. Apparently, only growth of the first four years is susceptible. For example, in the 5-year-age-class, in which the trees germinated from seed in 1921, the cankers have originated entirely in 1921, 1922, and 1923 internodes, the first three years' growth of the trees. In the 6-year-age-class in which the year of germination was 1920, three of the cankers started on internodes of 1923, the fourth year in the life of this class. This was the only instance in any one of the age classes of the occurrence of infection on the fourth year's growth. The rest of the infections were confined to growth put on during the first three years of the lives of the seedlings. The second year's growth was the most susceptible. The figures for cankers which started on such growth are inclosed by the diagonal lines in table 1. Of the total of 94 cankers, 54 started in such growth.

[1] Observations at other places indicate that the infection at Daisy Lake is typical.

TABLE 1.—*Growth affected by Cronartium comptoniae Arth. on Pinus contorta Loudon, Lake Lucile, Daisy Lake, B. C., November 1, 1925*
Analysis of 92 infections on 87 infected plants found in a total of 134 taken as a sample from a heavily infected patch of about 500 young pines

ge of d-ng rs)	Year germinated	Number of seedlings		Percentage infected	Number of cankers																			
					Of all ages											Young alone									
					Year's internode in which canker originated											Year's internode in which canker originated									
		Total	Infected		'25	24	'23	'22	'21	'20	'19	'18	'17	'16	Total	'25	'24	'23	'22	'21	'20	'19	'18	'17	Total
3	1923	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	1922	2	2	100	0	0	1	1	—	—	—	—	—	—	2	0	0	0	0	—	—	—	—	—	0
5	1921	13	7	54	0	0	1	3	3	—	—	—	—	—	7	0	0	0	0	0	—	—	—	—	0
3	1920	30	23	77	0	0	3	3	15	5	—	—	—	—	26	0	0	3	1	0	1	—	—	—	5
7	1919	32	23	72	0	0	0	0	5	17	2	—	—	—	24	0	0	0	0	1	1	1	—	—	3
3	1918	24	15	62	0	0	0	0	0	5	10	0	—	—	15	0	0	0	0	0	2	2	0	—	4
)	1917	17	12	71	0	0	0	0	0	0	7	6	0	—	13	0	0	0	0	0	0	0	1	0	1
)	1916	11	7	64	0	0	0	0	0	0	0	5	2	0	7	0	0	0	0	0	0	0	2	0	2
	1915	3	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1914	2	—	—	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1913	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
.....		134	89	66.4	0	0	5	7	23	27	19	11	2	0	94	0	0	3	1	1	4	3	3	0	15

Most of the cankers were well developed and had produced aecia more than once. It was clear that the large majority of them had been in existence for some years. Fifteen of the 94 cankers, however, were in the incipient or early stages of development and of relatively late origin. The occurrence of these young cankers is shown in the right-hand section of table 1. It will be noted that they are not confined to the youngest trees. They are, in fact, more or less generally distributed irrespective of age class.

As just stated, these cankers were all of a class in stage of development. There could have been hardly more than a year's difference in age. Three occurred on 1923 growth, and very probably originated that year, since an assumption of infection in 1924 would allow a very short period for incubation. The year 1923, therefore, marks the probable beginning date of all of the young cankers, although it is possible that a few originated from infection in 1922. Assumption of earlier years of origin is possible for cankers of this group on growth of earlier origin than 1922, but this would be stretching the incubation period beyond the limits of probability. Turning again to the left-hand section of table 1, reference to columns 10, 11, and 12 shows the majority of all the cankers, most of them relatively old and in well-advanced stages of developemnt, to be located on 1921-, 1920-, and 1919-growth. None of this majority could have originated prior to 1919, the largest share could not have originated prior to 1920, and 23 could not have originated prior to 1921. This, in general, indicates a relatively short incubation period.

The occurrence of young cankers on some of the oldest trees is therefore interesting, in view of the limitation of infection to the first four years' growth. It indicates that this growth continues to remain susceptible for a period of several years, while the nodes and internodes of more recent development, immediately adjacent on the same tree, are immune. This seems so odd a circumstance that the writer is inclined to question whether further investigations and a larger basis of observations will not show some susceptibility in nodes and internodes produced at least a few years subsequent to the fourth year in the life of the tree.

The degree and extent to which the growth of the first four years retains its susceptibility can not be read clearly from the present data. Obviously, the length of the period of susceptibility will depend, in large measure, on how infection takes place. The mode of infection is not known for *C. comptoniae*. It might be directly through the bark, through the needles, or both. In the first case, thickening and hardening of the outer bark would, probably, very soon prevent any penetration by the germ tubes of the delicate sporidia. In case infection occurs through the needles, the period of susceptibility might be longer. Young lodgepole pine retains a season's growth of needles commonly seven or eight and sometimes as many

as nine years. The opportunities for infection through this channel might remain at their maximum for 4 or 5 years. With the commencement of defoliation the possibility of infection would obviously decline and, on complete defoliation, a season's growth would be immune. With susceptibility limited to the growth of the first four years, the trees would become immune when the last needles dropped from the fourth year's growth, or, at the latest, in the thirteenth year of the life of the tree.

The figures for the occurrence of the young cankers, given in the right-hand section of table 1, show that susceptibility may persist for a number of years. Confining attention to the classes with the most substantial bases, i.e., the 5- to 10-year-age-classes, something of the degree of susceptibility during this time may be gathered from the figures on the percentage of infected trees per age class.

To interpret these figures, one should know approximately the year in which the disease first invaded the patch. The oldest growth on which cankers had originated anywhere in the entire infection area was that of 1917. The disease therefore did not enter prior to 1917. It is barely possible that it may have entered in 1917 or 1918. In this case, however, since trees of the 1914, 1915, and 1916 generations were present, it would be very difficult to explain the absence of cankers on wood grown in 1914 to 1916. Furthermore, the most advanced cankers on growth put on in 1917 and 1918 were entirely similar in stage of development to those in the most advanced stages on growth made in 1919 and 1920. Together, they form an approximately equal-age group representing the oldest infection on the area. Assuming that they were of exactly equal age, it would be obvious, since some of them occurred on growth made in 1920, that none of them could have originated from infection earlier than 1920. Allowing a year for age variation, part of them might have originated from infection in 1919. Comparison with the younger age classes of cankers made it plain that the large majority originated prior to 1921. The first infection of the pines, then, evidently must have occurred either in 1919 or 1920.

Assuming that the disease first became established in the patch six years before, or in 1919, trees in the 5- and 6-year-age-classes were then nonexistent. They must have become infected in later waves of inoculation. Trees in the 7-year-age-class were in their first year of growth. Those in the 8-year-age-class were in their second year. Obviously, being larger, they must have offered a larger inoculation target than did those in the 7-year-age-class and, so on, to the 10-year-age-class. With susceptibility limited to the first four-years' growth, the trees in this latter class, being in their fourth year, were then virtually at their maximum target size. They should normally have remained quite unchanged in this respect through the entire period of years in which the cankers originated, or from 1919 to 1924. If

the mode of infection is through the needles, there would have been some reduction of target in this age-class by the end of 1924, because of defoliation of the first two-years' growth. But the third- and fourth-year internodes were still only seven and six years old, respectively, and normally should still have had full quotas of needles, good for another season at least. Thus during the period of infection, 1919 to 1924, in the 5- to 10-year-classes, defoliation may be considered of negligible influence upon the chances for infection, and it is obvious that exposure to infection, both in degree and duration, must have been very much greater in the older than in the younger trees of these classes. On this basis, the percentage of trees infected and the number of cankers per tree should show a very decided increase in the older classes. The fact that there is no such increase is evidence of a rapid reduction in susceptibility considerably in advance of defoliation of the susceptible parts. Whether or not this indicates infection through the bark and resistance to be the result of early thickening and hardening of the outer layers or whether it indicates early development of resistance in the needles, can not be stated.

The extent of damage caused by the infection just described is evident in table 2.

Of the 134 trees, approximately 7, 4, and 13 per cent, respectively, are killed, dying, and badly stunted and deformed. Taken as percentages of the infected trees, these figures become approximately 10, 6, and 19. In the column headed "killed" the trees are entered in their proper age class. Although most of these trees died in the year of the examination, one or two had succumbed the year before. The age at death was, therefore, a little younger in several cases than indicated. It is evident from this column and from the data on the dying trees and on those that are badly stunted and deformed that the susceptibility to damage is highest in the youngest age classes and that it decreases rapidly with increasing age of the tree. Apparently the disease is not destructive of trees beyond the ages of 8 or 9 years.

If the seedling survives this early period of susceptibility, it rapidly develops further resistance to the effects of the disease, the disease is more or less completely overcome, and the external evidences of its presence largely disappear. Aecial production stops entirely and the only symptoms may be the remains of the witches' broom or killed leader of the original seedling at the base of the tree, more or less calloused over and enveloped or extruded in the subsequent growth, together with perhaps some evident abnormal swelling near the root collar. In some cases the swelling may extend irregularly up the bole for some distance, apparently indicating that the mycelium is still active in the bark of the slow-growing trees thus affected. At a place about 4 miles north of the

TABLE 2.—*Effects of Cronartium comptoniae Arth. on 134 plants of Pinus contorta Loud. selected as a sample lot from a heavily infected patch of about 500 young trees at Lake Lucile, Daisy Lake, British Columbia, Nov. 1, 1925*

Age of seedlings (Years)	Number of trees Basis	Infected		Killed		Dying		Others badly stunted and deformed	
		Number	Percentage	Number	Percentage of number infected	Number	Percentage of number infected	Number	Percentage of number infected
4	2	2	100.00	1	50.00	—	—	1	50.00
5	13	7	54.00	3	42.80	1	14.30	1	14.30
6	30	23	77.00	3	13.05	3	13.04	7	30.40
7	32	23	72.00	1	4.35	1	4.35	6	26.04
8	24	15	62.00	1	6.66	—	—	1	6.66
9	17	12	71.00	—	—	—	—	1	8.33
10	11	7	64.00	—	—	—	—	—	—
11	3	—	—	—	—	—	—	—	—
12	2	—	—	—	—	—	—	—	—
Total	134	89	66.40	9	10.10	5	5.62	17	19.10
Percentage of the total basis					6.72		3.73		12.68

infection center at Lake Lucile, within 100 to 150 feet of large thickets of *Myrica gale* found well infected with the rust on October 24, 1925, four trees were found which bore striking evidence of the capacity of *Pinus contorta* to overcome the attack. These trees were 22, 24, 35, and 37 years of age. In the presence of the old, dead witches' brooms and killed parts of the original seedlings at their bases, all bore distinct evidence of earlier infection by *Cronartium comptoniae*. There was nothing else in that section which could very well have caused such symptoms and the situation of the trees with respect to the sweet gale and the presence of two younger trees, 9 and 14 years old, in the early stages of recovery from the disease, left little room for doubt as to the diagonsis. The effects of the disease in two of the four trees were practically negligible. In the other two, growth was slower than normal and on the lower bole of one of these the irregular swelling just mentioned was conspicuous.

The evidence of infection on these trees indicated that the rust had been present in this locality some thirty years or more. No doubt some of the other trees of similar age and possibly others of still greater age in the locality, which now show no evidence of the disease, had undergone a similar juvenile attack.

PRODUCTION OF FRUITING STAGES AND METHOD OF SPREAD

On the youngest trees where *Cronartium comptoniae* is in full activity, aecial sporulation occurs mainly in the spring during late April and May. Production of a few small aecia may occur also in late fall, depending upon the character of the season. This seems to be a rather common occurrence with Cronartium in the Pacific Northwest. The writer has noted it regularly with *C. ribicola* in this territory as well as in several instances with *C. filamentosum*. The uredinial stage appears on *Myrica gale* usually in June and July, the telial stage following in abundance in August and to the dropping of the leaves.

So far, the disease has been found wherever sweet gale has been examined in the fall. In most places trees of *Pinus contorta* have occurred in the immediate or near vicinity and the rust has been transmitted to the neighboring sweet gale from them. In a number of localities, however, this is not the case. Nevertheless, infection of the sweet gale in those localities has been plentiful in the fall.

The presence of the rust in these cases and its general occurrence in all the scattered places in which sweet gale has been found seem to indicate, as with *Cronartium ribicola* in the West (14, 11), that this rust may spread great distances from the aecial to the uredinial hosts. Other possible methods of spread are eliminated through lack of any affirmative indications. Among the possibilities may be considered the same factors that have been

considered in arguments for the method of spread of *C. ribicola,* such as (1) overwintering of the rust in the uredinial stage and spread by urediniospores, (2) direct transmission of infection from pine to pine by means of aeciospores, and (3) spread by means of sporidia. With respect to overwintering in the uredinial stage, there is not the least evidence that this occurs in the Pacific Northwest. *Myrica gale* loses its leaves entirely each fall and evidently the rust goes with them. No signs of infection on other parts of the plant, such as the shoots and buds, which might carry the disease over the winter, have been noted. And even where sporulating aecia have been situated in immediate proximity to the sweet gale, it has not been until well past full leaf development that the first signs of uredinial infection have been observed to make their appearance in the following spring.

With respect to the possibility of direct aecial spread or of wide spread by means of sporidia, the rust on pines has been invariably found only within very limited radii of the sweet gale. Heavy pine infection in the immediate vicinity of the latter host rapidly gives way to sparse and light infection as the distance from the sweet gale increases, and beyond a distance of two- or three-hundred yards becomes negligible. The concentration of pine infection within such short distances of the uredinial host argues quite evidently against any supposition of direct aecial spread or long-distance spread by sporidia to explain the extent and characteristics of the distribution noted. It is possible that birds and insects may have played a considerable part in distributing the disease either by dissemination of aeciospores or urediniospores, although there seems to be nothing particularly attractive to either of these classes of potential spore carriers about either of the hosts. This seems especially to obtain with the sweet gale which has shown little or no evidence of insect attacks and is apparently little frequented by birds.

Another possibility may be that of wind dissemination of the urediniospores. Urediniospore production, however, is light as compared with the production of aeciospores which are carried away in clouds at the least jar of the infected pines during sporulation in the spring, and all indications seem to suggest rather that wide dispersal of the rust is accomplished mainly by wind dissemination of the aeciospores.

The disease has been found quite to the southern limit of occurrence of the uredinial host. The sweet gale ranges north into Alaska, east to Labrador, and south into the known range of the rust in the East. Susceptible pine hosts are distributed transcontinentally over the same territory. It is probable therefore that the rust spans the continent on the north and that its eastern and western ranges are connected.

No associations of *Myrica gale* with *Pinus ponderosa,* the most important timber tree of all the western hard and pitch pines, have yet been noted

within the natural range of this pine. Consequently, no occurrence of the disease within this range has yet been recorded. With evidence that it is highly susceptible, however, (13) and in view of the indications of the rust's capacity for long-distance spread it may be expected that infection will be found on it here, also, where such associations occur. On the other hand, observations made to date, together with distribution data from various floras, indicate that these associations are rare, if they exist at all. Consequently the rust can not be considered a serious threat to western yellow pine within the native range of this tree.

It was at first thought possible that *Myrica californica* Cham. & Sch. might be a factor in carrying the rust southward from the southern limits of *M. gale* into the southern Oregon and California pitch- and hard-pine regions. While this species of Myrica had been shown by inoculations in the East (20) to be immune from the disease, and scouting for the rust on it in California by Meinecke and his associates had resulted negatively, there still was some question as to whether, under natural conditions, it might not become infected. Opportunity to check up on this was afforded at Ilwaco, Washington, near the southern limits of the occurrence of *M. gale*. The disease was found here on *M. gale* and *Pinus contorta* in the immediate vicinity of *M. californica*. The latter showed no sign of uredinia or telia and it may be concluded that this species, as indicated by the results of eastern inoculations and of previous western scouting, will be of no significance in the rust's distribution.

SUMMARY

An account is given of the discovery of the rust disease caused by *Cronartium comptoniae* in the Pacific Northwest. Its range includes the section west of the Cascade Mountains from Ilwaco, Washington, north to Prince Rupert, British Columbia. The disease is indigenous here, as in the East. The only known uredinial host of the rust within this range is *Myrica gale*. The aecial stage has been found only on *Pinus contorta*.

The uredinial host is a plant of very scattered occurrence in bogs and along lake margins where it is of little or no value, inaccessible, and easily overlooked. The aecial stage of the fungus closely resembles another indigenous and much more widely distributed rust, *C. filamentosum* which completely overlaps its range. These two factors contributed largely to the fact that the disease was not discovered earlier in the West.

In the fall, a wide distribution of the disease has been noted on *M. gale*, irrspective of the proximity of the aecial hosts. This distribution is attributed to long-distance dissemination of the rust by wind-borne aeciospores.

Both the aecial and the uredinial hosts occur transcontinentally between the northern limits of the known ranges of rust in the East and the West.

It is probable, therefore, that the disease also is transcontinental and that the eastern and western ranges of it are connected.

The disease is destructive to young *Pinus contorta* in the near vicinity of sweet gale. As high as 10 or more per cent of the seedlings may be killed in such situations. Studies indicate that nodes and internodes originating in the first four years of the life of the seedling only are sufficiently susceptible for the establishment of infection. Many of the trees have the faculty of largely recovering from the disease. Possible modes of infection and evidence on the development of resistance of the host are discussed.

No associations of the alternate host with *Pinus ponderosa,* which has shown itself to be highly susceptible in eastern nurseries, have been found. Such associations probably are rare, if they exist at all. Consequently, the rust can not be considered a serious threat within the native range of this pine.

Observations on *Myrica californica* at Ilwaco corroborated results of inoculation by others in the East, showing this species of Myrica to be immune. The fact that it ranges southward into the Oregon and California pitch- and hard-pine regions may be concluded to be of no significance in connection with the possible distribution of the rust into these regions.

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ECOLOGICAL STUDIES OF CURLY TOP OF SUGAR BEETS

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ONE FIGURE

Studies on the ecology of *Eutettix tenellus* (Baker), during 1926, included a consideration of the environmental data from beet fields and covered the factors of evaporation, temperature, and light. Inasmuch as that year was a serious one for the sugar-beet industry in southern Idaho, an excellent opportunity was presented for study of these factors in the development of curly top.

Concurrent with these studies, an attempt was made to modify, by artificial means, factors of temperature, light, and moisture loss from the leaves under field conditions.

STATEMENT OF THE PROBLEMS

1. The development of curly top under artificial shade in the field and various light sources in the laboratory.

2. The effect of artificial control of environment on the development of curly top.

METHODS

Problem 1. Cages were constructed of three materials—(*1*) automobile celluloid, with the ends of the cages of a fine cloth mesh for ventilation; (*2*) "Celoglas" (a glass substitute), with ventilation provided by means of strips of cloth sewn into both ends and top; and (*3*) unbleached muslin. Two laboratory light boxes were made, one lighted by a quartz mercury-arc lamp (Uviarc Poultry Treater) under which a thin Pyrex-glass bowl was placed, and the other lighted by a 1000-watt clear Mazda lamp. Under this type of lamp, it was necessary to have a glass-bottom chamber through which water was constantly running in order to reduce the temperature.

Problem 2. Hydrated lime, zinc oxide, and lamp black were sprayed on the leaves of the beets. Difficulty was experienced in getting heavy suspensions through the sprayer, but by modifying the nozzles it was possible to use the formulae given below:

Lime—hydrated	50 lbs.	Lamp black	8 lbs.
Calcium caseinate	3 lbs.	Calcium caseinate	3 lbs.
Water	30 gal.	Water	30 gal.
Zinc oxide	40 lbs.		
Calcium caseinate	3 lbs.		
Water	30 gal.		

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Measurement of factors. Light intensity was measured by comparison with a Kimball Sunshine Recorder (3). This measures total intensity and is not selective for the various wave lengths. For the laboratory chambers, the recorder was placed under the lights at the same distance from the lights as were the plants. For the outdoor cages, the record was taken by placing each cage consecutively over the recorder and noting the changed deflection of the needle. These data are listed in table 1. Air temperature was measured by means of "distance recording" thermographs. Humidity, or rather the evaporating power of the air, was measured by means of Livingston porous-cylinder atmometers.

THE DEVELOPMENT OF CURLY TOP UNDER ARTIFICIAL SHADE IN THE FIELD AND VARIOUS LIGHT SOURCES IN THE LABORATORY

Experiments were conducted with the three types of cages mentioned; the ultra-violet and 1000-watt clear Mazada lamps were also used in two of the experiments. The sunshine recorder was not available at this time, but later readings, as given in table 1, show that light intensity was greatest under celluloid, less under Celoglas, and least under cloth. In all these experiments the same number of leaf hoppers, taken from a very badly diseased field, was used, but these were not individually tested for virus bearing. The plants, which were of the same age and from the same lot of seed, were continually exposed to the insects during the whole period of each experiment.

The results of these experiments indicate that a greater percentage of plants developed the disease under celluloid and Celoglas than under cloth or the two types of artificial light. Differences in the severity of the disease were also marked. Under celluloid and Celoglas, pronounced curling and vein swelling were general, while under the cloth cage the symptoms did not, as a rule, develop beyond the clearing of the veins. None of the cases developing under the artificial lights ever progressed beyond that stage.

In another experiment, 15 plants were severely etiolated by growing them in a dark basement at 85° F. for six days. Outdoors 15 other plants were placed in a celluloid cage for the same period. At the end of this period five leaf hoppers were placed on each of the 30 plants and left there. Three days later the etiolated plants were removed to another, similar celluloid cage.

The result of this experiment showed only three cases of curly top in the etiolated set as against the 15 plants in the set kept outdoors previous to infection. All the cases of the disease in both sets were of the pronouncedly curled and vein-thickened type. This experiment indicates that the condition to which the plant is exposed at the time of inoculation affects the

TABLE 1.—*The intensity of sun's rays in the open and under cages of different materials and the intensity of two artificial lights measured by means of the Kimball Sunshine Recorder*

Sunlight				Artificial light	
In the open	Under Celoglas	Under celluloid	Under unbleached muslin	From 1000-watt Madza lamp	From ultra-violet lamp[a]
100	70	100	50	15	2
70	49	70	—	—	—
100	70	100	—	—	—
52	30	52	16	—	—
49	—	44	—	—	—

[a] Uviarc Poultry Treater with Pyrex screen.

number of positive cases even if conditions later are favorable to the development of the disease, but these results were not checked by further experiments. It might well be that conditions affecting susceptibility to infection are not identical with those affecting the development of the disease.

Additional experiments were conducted in the three types of outdoor cage in which the factors of light, temperature, and evaporation were measured in the manner previously described. The environmental data for these experiments are listed below in tables 2, 3, and 4.

In one experiment (see table 2), five leaf hoppers were caged on each plant and 10 plants used for each of the following conditions: the three outdoor cages, the ultra-violet and 1000-watt lamps, and also a temperature box kept at 85° F. No light was available in this last case.

Not a single case of disease developed in either the dark temperature box or under the ultra-violet light. Three cases of curly top developed under the 1000-watt lamp, of which one was of the curled and swollen-vein type. In case of the three outdoor cages, all the plants under celluloid developed disease of the pronounced type; in addition, the plants were badly stunted. All the plants under Celoglas developed disease of the same type, but stunting was not so pronounced. Under the cloth cage only three developed symptoms of the severe type though there were nine cases in all.

It should be noted that in this experiment the development of the disease varied directly with the amount of the three factors, light intensity, evaporation, and temperature, disease being most pronounced where these three factors were at a maximum. The light intensity was extremely low under the ultra-violet light, and was altogether lacking in the temperature

TABLE 2.—*The effect of light, evaporating power of the air, and temperature on the development of curly-top symptoms in sugar beets. Experiments made at Twin Falls, Idaho, July 8 to August 4, 1926*

Data recorded \ Outdoor cages and artificial lights		Celluloid	Celoglas	Cloth	1000-watt Mazda lamp	Ultra-violet lamp	Temperature box at 85° F.
Mean daily evaporation in cc.		36.4	34.8	30.00	——	——	——
Mean temperature in degrees F.	Maximum	110.0	109.0	98.0	——	——	85° F.
	Minimum	55.00	56.0	56.00	——	——	85° F.
Light intensity in gr. cal. per sq. cm.	Total	15,298.70	10,070.90	7,649.35	5,832.93	548.0	——
	Mean daily	611.5	428.3	305.7	216.04	20.3	——
No. plants with curly-top symptoms		10	10	9	3	None	None
Type of disease		All severe and plants stunted	All severe, but very little stunting	3 severe cases; 6 mild; no stunting	1 severe case; 2 mild; no stunting		

TABLE 3.—*The effect of light, evaporating power of the air, and temperature on the development of curly-top symptoms in sugar beets. Experiments made at Twin Falls, Idaho, July 21 to August 16, 1926*

Data recorded \ Outdoor cages and artificial lights		Celluloid	Celoglas	Cloth
Mean daily evaporation in cc.		29.6	27.2	27.0
Mean temperature in degrees F.	Maximum	111.0	108.0	106.0
	Minimum	56.0	54.0	52.0
Light intensity in gr. cal. per sq. cm.	Total	16,338.90	11,237.30	8,169.46
	Mean Daily	605.1	439.4	302.5
No. plants with curly-top symptoms		4	4	3
Type of disease		3 severe cases; 1 mild case; stunting very pronounced.	2 severe cases; 2 mild; vein thickening most pronounced. Some stunting.	All mild; no stunting or vein thickening.

box. This same factor was low, compared with the outdoor cages, under the 1000-watt lamp.

In another experiment (see table 3) which was limited to field cages, five *E. tenellus* were placed on each plant. Ten plants were placed under each of the celluloid and Celoglas cages and eight plants under the cloth cage. Growth was most vigorous under cloth, less in Celoglas, and least vigorous in the celluloid cage. Stunting was most pronounced in the celluloid cage, but the curling and vein thickening of the leaves was most conspicuous in the Celoglas cage. The three cases of disease which developed in the cloth cage were all of the faintest type.

TABLE 4.—*The effect of light, evaporating power of the air, and temperature on the development of curly-top symptoms in sugar beets. Experiments made at Twin Falls, Idaho, August 25 to September 13, 1926*

Data recorded \ Outdoor cages and artificial lights		Celluloid	Celoglas	Cloth
Mean daily evaporation in cc.		26.0	23.8	24.0
Mean temperature in degrees F.	Maximum	103.0	105.0	92.0
	Minimum	48.0	48.0	44
Light intensity in gr. cal. per sq. cm.	Total	13,110.0	9,175.0	6,555.0
	Mean Daily	524.4	367.0	262.2
No. plants with curly-top symptoms		9	8	8
Type of disease		7 severe cases; 2 mild; stunting pronounced	7 severe cases; 1 mild case; very little stunting.	6 severe cases; 2 mild; yellowing and stunting very pronounced.

In the third experiment (see table 4) no difference could be observed in the type of disease which developed. This was the last experiment of the season and the evening temperatures were low. The sun's intensity was much diminished, so that the total intensity under the cloth cage was brought down to the point where it probably was too low for normal growth. The plants in this cage were yellowed as well as stunted.

THE EFFECT OF ARTIFICIAL CONTROL OF ENVIRONMENT ON THE DEVELOPMENT OF CURLY TOP

Makers of paints and varnishes have devoted considerable attention to the effects of environment in producing "weathering," and it was after

reading a paper by Harley A. Nelson (4) that the writer conceived the idea that possibly an application of some light-screening pigment to the leaves of the sugar beet would have some effect on the development of the disease and the consequent weight of the beet at harvest time.

G. F. A. Stutz (6) describes the absorption coefficients of various paint pigments. Table 5 gives these data, and it was on the basis of these that the materials for this experiment were chosen.

There are no data available for the hydrated lime which was used, but so far as is known this material has no capacity to screen out the shorter wave lengths of the light spectrum.

TABLE 5.—*The absorption coefficients of two paint pigments. Taken from the work of Stutz (5, p. 92–93)*

Pigment	Coefficient of diffuse reflection at wave lengths							
	5461	4358	4047	3655	3131	3024	2968	2536
Zinc oxide	97	87	80	3	0	0	0	0
Lamp black	5	4.5	3.8	4.2	4.2	4.3	4.5	4.4

Builders' ordinary slaked lime and hydrated lime were also used. The former had to be abandoned owing to the impossibility of getting a mass finely enough divided. The hydrated lime worked through the sprayer very well, but with the weight of material used, frequent cleanings of the machine were necessary. The experimental plots of The Amalgamated Sugar Company at Twin Falls, Idaho, were used for this experiment. Two places were chosen, each with a different planting and thinning date. In the case of one plot, the spray was applied before thinning. The data for this experiment are shown in table 6.

Differences in dates for these sprayings were due to practical considerations. Each type of spray was applied as near the same date as possible. Plot 2 was planted after the migration of leaf hoppers into the fields had occurred, but the numbers of *E. tenellus* in this plot indicated that there had been a decided movement from surrounding plots.

The experiments during 1926 were conducted under as severe curly-top conditions as could be imagined. In order to compare the results with those obtained when curly top was not a factor, experiments were conducted on small plots during 1927. In the Twin Falls area in 1927, beets made remarkable growth and were only slightly affected by curly top, so the results for that season can be taken as representing the effect of the pigment sprays under normal conditions. Spray was applied on June 24, July 2, July 20, and August 5. The harvest data are shown in table 7.

TABLE 6.—*The effect on yield of applications of light-screening pigments to sugar-beet leaves*

Plot 1. (*Planted April 8, thinned May 10, 1926*)

Spray material	Dates of spraying	No. plants in row at beginning of exp.	No. beets harvested	Beets which weighed 1 lb. or more		Beets weighing 8–15 oz.	Total weight of beets (lbs.)
				No.	Weight (lbs.)		
Zinc oxide	June 1, 10, 19 and July 6	145	125	17	.....	15	48.0
Check		143	129	24	.....	22	53.9
Lamp black	June 5, 9, 24, 30 and July 15	155	114	7	.....	18	30.6
Check		141	132	24	.....	19	63.4
Lime (hyd.)	June 5, 8, 23 and July 8	142	134	33	.....	19	82.0
Check		154	147	18	.....	26	64.7

Plot 2. (*Planted May 17, thinned June 25, 1926*)

Spray material	Dates of spraying	Size of plot	Beets which weighed 1 lb. or more		Beets weighing under 1 lb.		Total weight of beets (lbs.)
			No.	Wt. (lbs.)	No.	Wt. (lbs.)	
Lime	June 11, 26 and July 6, 17	6 rows 300 feet long	144	207.5	1163	473.5	681.0
Check		do	66	107.0	1091	376.5	483.5
Lamp black	June 13, 27 and July 10	do	28	42.0	965	294.0	336.0
Check		do	26	40.5	774	307.0	416.0
Zinc oxide	June 17, 28 and July 9	do	38	57.25	843	310.0	367.25

TABLE 7.—*The effect on yield of applications of light-screening pigments to sugar-beet leaves. Data taken from experimental plots in 1927*[a]

Pigment	Total yield of beets (lbs.)	Pigment	Total yield of beets (lbs.)
Zinc oxide	620	Check	695
Hydrated lime	690	Check	700
Lamp black	340	Check	610

[a] Each plot consisted of two rows of beets 180 feet long.

During the summer, occasional records were taken of the surface temperature of normal and of treated leaves. This was done by means of a thermocouple which was held close to the surface of the leaf, the operator avoiding interference with the sun's rays and keeping at arm's length from the plant. These readings revealed the fact that the surface temperature of all the leaves tested, sprayed and unsprayed, were similar to within a degree Centigrade, even though the leaves of the treated plants presented two entirely different absorbing surfaces, white of the zinc oxide and lime and black of the lampblack.

In a recent paper, Angstrom (1) gives data on the solar intensity transmitted through leaves of various species. He obtained these data by carefully fastening the leaves over the glass globe of a sunshine recorder of the thermoelectric type and recording the light transmitted. Using the same method, beet leaves were fastened over the globe of a Kimball pyrheliometer (fig. 1) which was then placed under a 100-watt Mazda lamp. After the record of transmission through the normal leaf was obtained, the leaves

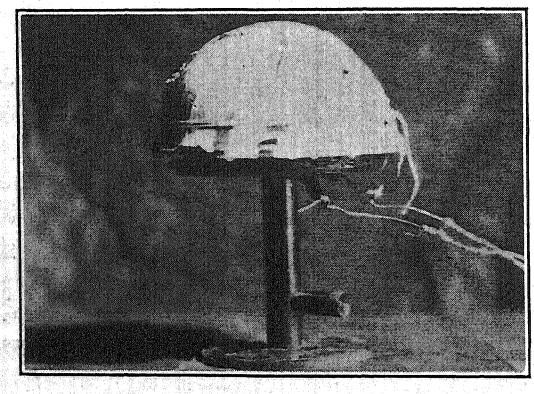


FIG. 1.—Kimball pyrheliometer, showing method of obtaining transmission through beet leaves. The glass globe is covered with beet leaves carefully fastened together and, in the case figured, coated with lime spray.

were sprayed with lampblack of the same density as that used in the experiments. The record was again obtained. This was done for each of the three pigments, using a new set of normal leaves in each case. These data are presented in table 8.

TABLE 8.—*Data on transmission of light through sprayed and unsprayed beet leaves*

Light transmission of unsprayed leaves		Light transmission of sprayed leaves		Reduction in light transmission by foliage spraying (per cent)
Unsprayed No. 1	31[a]	Lampblacked	10	68
Unsprayed No. 2	38	Zinc-oxided	23	40
Unsprayed No. 3	34	Lime	22	36

[a] Reading direct from chart for purposes of comparison only.

The three pigments used vary considerably in their capacity as light screens. Both lampblack and zinc oxide are efficient in screening out the shorter wave lengths of the spectrum. Both zinc oxide and lime, being white, reflect the longer wave lengths, whereas lampblack absorbs them. Lime has little capacity as a screen against the short wave lengths.

The severe reduction in yield of plants sprayed with lampblack was common to both seasons, 1926 under severe curly top conditions and 1927, when curly top was not a factor. It is evident, then, that the pigment seriously affected the normal functions of the plant. This interference was probably with the three factors of temperature, moisture loss, and light. Clum (2) concludes that leaf temperatures depend upon a balance between light intensity and convection and radiation, but considers transpiration insignificant as a factor in reducing temperature, at least in Ithaca, N. Y. The fact that the plants sprayed with lampblack wilted down when all other plants in the experiments were not so affected, supports the view that moisture loss was very greatly increased through the absorption of heat by the black surface; the reduction of the temperature of the leaf surface to that of normal beets is evidence of the plant's response.

The data from the plots which were sprayed with zinc oxide indicate a slight reduction in yield in both seasons. Since the plant was protected from extreme heat by its white heat-reflecting cover, this reduction could not have been due to abnormal moisture loss. Photosynthesis was probably seriously interfered with; however, the possible toxic effects of the zinc oxide have not been investigated. In the case of the other two pigments used, toxicity of the material is unlikely, in view of the fact that one consists of finely divided carbon and the other of an inert carbonate.

The results from the plots sprayed with hydrated lime are most interesting, since in the case of this material there is a significant difference between the yields of the two seasons. In 1926, when curly top was a serious factor, the plot sprayed with hydrated lime was superior in yield to its check. In 1927 there was no significant difference between it and its checks, and in that season curly top was barely present in the plots. This result suggests that the development of curly top was conditioned by the presence of the lime screen on the leaves. As has been mentioned, lime is a very inefficient screen for the shorter wave lengths of the spectrum but provides a white reflecting surface. There was, then, very little interference with photosynthesis and at the same time moisture loss was reduced, owing to the reflection of heat from the leaf's surface. Since it has been shown in the first section of this paper that extreme conditions of temperature, evaporation, and solar intensity favor the development of the disease, the explanation that the effect of the hydrated lime spray was through reduction of the intensity of these factors is at least plausible.

The relative importance of the three factors mentioned is difficult to determine, especially as extremes of one are usually associated with similar extremes in the others. Certain field data are available through the courtesy of the Amalgamated Sugar Company. These data are presented in table 9, and are concerned with the course of the disease and its severity in a beet-growing area which includes considerable subirrigated land.

TABLE 9.—*The effect of different types of irrigation on the yields from sugar-beet fields in which curly top was present*

Yield	Subirrigated fields	Surface-irrigated fields
Not harvested	47.50%	86.98%
5–9 tons	36.66%	12.06%
Over 9 tons	15.75%	0.95%

These data illustrate the fact, well known to workers on curly top, that land kept moist and therefore cool, permits beets to withstand more easily the development of the disease. Presumably the evaporating power of the air just above the subirrigated beets was considerably less than over beets irrigated in the ordinary way; but, even if this was not the case, subirrigated beets do not suffer from drought in the early stages and this fact no doubt contributed to the vigor of these beets. It has been the common experience in this subirrigated area that even in the years of worst curly-top infestation, many fair and some good yields have been obtained. No subirrigated land exists near the Twin Falls factory, but it is perhaps significant that the only field producing 10 tons to the acre in 1926 was underlaid

by a leaky pipe line. The position of this pipe line could easily be traced across the field, since the beets over it were of large size and in healthy condition.

Field observations on the effect of low light intensity in retarding the development of the disease are numerous, but since this factor is invariably associated with low intensities of the factors of temperature and moisture loss it has not been possible to evaluate its individual importance. Shapovalov (5) came essentially to the same conclusions as to the importance of these factors in his studies on western yellow blight of tomatoes, a virus disease of the same origin as curly top.

SUMMARY AND CONCLUSIONS

The development of curly top appears to be more severe under conditions of high light intensity, temperature, and evaporation than under conditions where these factors are reduced in intensity. The number of positive cases appears to be greater under conditions of high light intensity, temperature, and evaporation. One experiment indicates that the conditions governing susceptibility to infection are not the same as those conditioning the later development of the disease. Experiments on the control of the external environment indicate that lampblack and zinc oxide, when sprayed onto the leaves, interfered with the normal functions of the plant and did not favor the development of the disease. Hydrated lime reduced the severity of the disease but did not affect the normal beet.

The kindness of Drs. Burton E. Livingston and H. H. Kimball in answering questions concerning their particular fields is gratefully acknowledged. Dr. Eubanks Carsner, of the Bureau of Plant Industry, has reviewed the manuscript and made valuable suggestions in connection therewith. Messrs. C. H. Griffith and V. E. Romney assisted with the field work during the entire experiment.

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NEMATOSPORA ON POMEGRANATES, CITRUS, AND COTTON IN CALIFORNIA[1]

HOWARD S. FAWCETT

The purpose of this paper is to give a brief report of observations and experiments with respect to a Nematospora found on pomegranates and citrus in Imperial County, California, in September, 1928, and, later, found widely distributed in the same county on cotton bolls.[2] This fungus probably is *Nematospora Coryli* Peglion. The Western leaf-footed plant bug, *Leptoglossus zonatus* Dallas was found to be carrying the fungus from the pomegranate to citrus. This appears to be the first recorded occurrence of this fungus on the three above-named hosts in the United States. The pomegranate appears to be a new host for the fungus and this plant bug is a newly reported carrier of the fungus. Accepting Ashby and Nowell's (1) conclusions that *Nematospora Coryli* also includes *N. phaseoli* Wingard (2) and *N. lycopersica* Schneider (3), the only other reports of its occurrence in the United States appear to be that on beans in Virginia and that on tomatoes in southern California.[3] It has been reported by Nowell (1, 4) on citrus, cotton, and fifteen other hosts in the Lesser Antilles. It was reported by Lee (5, 6) on citrus in the Philippine Islands, Japan, and China, and recently by Rhind (10) on cotton in Burma, India.

Another quite distinct species, *N. gossypii,* has been described recently by Ashby and Nowell (1) as having been found on cotton and on two other hosts in the Lesser Antilles. The last species also has been found on cotton in Nigeria, Africa (7).

In general, *N. Coryli* causes a staining and collapse of the tissue, resulting ultimately in desiccation and frequently in a dry rot. In pomegranates there first appear depressed, light-colored spots in the fleshy covering surrounding the seeds. These later develop into a general browning and collapsing of certain regions of the pulp involving the fleshy part of many adjacent seeds. In citrus there is a collapsing and drying out of the juice sacs just inside the rind. The effect noted on oranges, grapefruit, and tan-

[1] Paper No. 203, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Plant Disease Reporter, Bureau of Plant Industry, U. S. D. A. 12: No. 14, pp. 145–146. December 1, 1928.

[3] Schneider, in Phytopathology 6: 395, says that the first tomatoes examined were obtained from a restaurant in Berkeley, California, whose proprietor maintained that they were from the South Sea Islands, but the author thought it more likely "that they came from Mexico or perhaps from Cuba." In Phytopathology 7: 52, it is stated under *Habitat:* "Parasitic on nearly ripe and ripe fruit of *Lycopersicum esculentem,* southern California, Cuba and Mexico."

gerines in the Imperial Valley, California, was a brownish to reddish brown stain in a portion of each of the juice sacs attacked. In time this results in a drying out and deterioration of a considerable portion of the pulp without any spotting or deterioration on the exterior of the rind (Fig. 1).

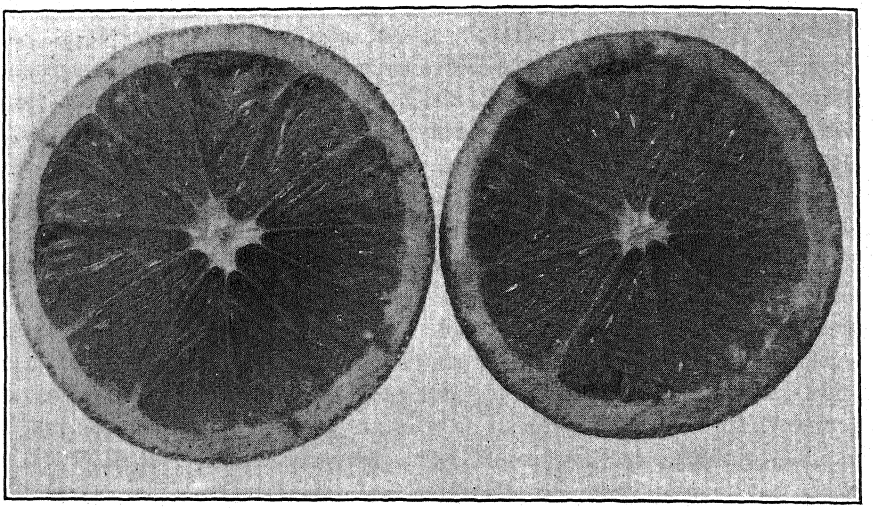


FIG. 1.—Oranges showing breaking down and drying of juice sacs due to *Nematospora Coryli* introduced through punctures of *Leptoglossus zonatus*.

If immature fruit is punctured by the bugs carrying this fungus, it begins to color prematurely and tends to drop from the trees. In the Philippine Islands, Lee (5) found, in addition to the drying out, a marked thickening of the divisions. In young cotton bolls the seeds are discolored and the inside portions become brown and dry. The immature lint was at first yellowish and later dark reddish-brown and dry, this discoloration and drying spreading various distances from the original punctures.

Experiments showed that the disease could be transmitted readily by placing leaf-footed puncture bugs, *Leptoglossus zonatus*, found on infected pomegranates, onto sound oranges and lemons in jars in the laboratory. In two or three weeks the symptoms developed on the inside of the fruit. One of the first indications of the presence of the disease in green fruits was a much more rapid yellowing on those which had been punctured by the bugs than on fruit not punctured. A fungus was isolated readily on glucose-potato agar and on corn-meal agar by transferring juice sacs with sterile instruments to the surface of slants. The growth of the California strain obtained from citrus on glucose-potato agar first resembled that of yeast. With age the growth tended to form an irregular and somewhat wrinkled surface resembling that illustrated by Ashby and Nowell (1). On corn-

meal agar the fungus grew much slower, with a considerable margin of mycelium-like growth on the surface of the medium.

Attempts were made to isolate this fungus from the beaks of several individuals of *Leptoglossus zonatus* but no Nematospora was obtained. *Aspergillus niger,* however, which also was being carried from pomegranates to citrus by these bugs, was readily isolated. Microscopic examinations of the beaks and other parts of the insect failed to reveal Nematospora, but showed many spores of Aspergillus adhering to the sides of the beaks. The small, yeast-like cells of Nematospora might be carried in small numbers without being easily identified under the microscope.

Experiments showed that the Nematospora, when transferred from pure culture by needle punctures into immature Navel oranges, would cause these fruits to turn yellow more quickly than those punctured with a sterile needle. Lee (5) already had shown that the Philippine fungus would infect citrus fruits when transferred to it by needle punctures. The California strain was found to grow very well on the glucose-potato agar at temperatures from 15 to 32° C. The growth was very slight even in 30 days at a temperature of 8.5° C. During a 10-day period its growth was greatest at 25.5 and 27.5° C. At all temperatures tried between 15 and 32° C. there occurred an abundance of asci and spores. Although different temperatures produced no striking differences in the general macroscopic appearance of the growth, they had a decided influence on the length of the asci. The following measurements were typical of the length of the asci found at different constant temperatures:

21.5° C., 72– 80 μ
24° C., 80– 93 μ
25.5° C., 88–102 μ
27.5° C., 104–106 μ

The width, varying between about 8 to 12 μ, did not appear to be related to temperature. The spores were apparently the same length (approximately 40 x 2.5 μ) irrespective of the length of the asci.

It is suspected that the fungus has been present in southwestern United States and Mexico for a long time, as indicated by descriptions of injury to cotton bolls in previous entomological literature. Morrill (8), in 1910, described an injury to cotton bolls in Mexico and Arizona, strikingly similar to the Nematospora injury which the author has recently observed where Nematospora is abundant. McGregor (9), in 1927, referring to some work done from 1916 to 1918, in the Imperial Valley, makes the following statement regarding the tarnished bug, *Lygus elisus* Van Duzee: "Since the actual mechanical injury associated with the feeding of Lygus is slight, compared with the resulting damage, it seems reasonable to suspect that

either a toxic material or an organism may be introduced into the plant tissue at the time of feeding."

The descriptions and illustrations by these two authors of the more severe phases of the damage strongly indicate that Nematospora or other similar fungus has been for a long time a factor in the injury. This suspicion is strengthened by the report of recent experiments by Laycock and Jones (7) with the cotton stainer in connection with *Nematospora gossypii* in Nigeria, where very slight damage was produced by the punctures of bugs bred under sterilized conditions as compared with punctures from those known to carry the fungus.

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A PROGRESS REPORT ON THE CONTROL OF CROWN GALL, HAIRY ROOT, AND OTHER MALFORMATIONS AT THE UNIONS OF GRAFTED APPLE TREES[1]

A. J. Riker, G. W. Keitt, and W. M. Banfield

An effort has been made to find a satisfactory control of the various malformations which occur in the nurseries at the unions of apple trees grown from piece-root grafts. Following the work of Smith, Brown, and Townsend (10), all these malformations, except those caused by insects, have been classified by most workers either as crown gall or hairy root, and attributed to the crown-gall organism, *Bact. tumefaciens* Smith and Town. More recently a series of papers by Brown (1), Muncie (5), Riker, Banfield, Wright, and Keitt (7), and Riker and Keitt (9) have presented evidence that the problem is concerned not only with infections by a single species of bacteria, but rather with those of at least two species of bacteria and with certain non-parasitic troubles. In view of the complexity of the problem, it is not surprising that adequate control measures have developed slowly.

The control measures thus far tried fall into three major groups: (a) use of antiseptics applied to the soil, to the wrappers employed, or to the finished graft (Hedgcock, 2; Melhus and Maney, 3; Oppenheimer, 6; Sherbakoff, 11; Waite and Siegler, 12; and Wormald and Grubb, 13); (b) modifications[2] of the grafting methods (Hedgcock, 2; Melhus, Muncie, and Fisk, 4; and Riker and Keitt, 9); and (c) modifications in wrapping materials and methods (Hedgcock, 2; Riker, Banfield, and Keitt, 8; and Riker and Keitt, 9). The problem of control has been approached by the writers in all these directions in a long series of experiments during the past five years. While various methods have given promise in certain individual experiments, only one of them in the writers' trials has been found to give consistently favorable results over a considerable range of conditions (Riker, Banfield, and Keitt, 8; Riker and Keitt, 9). This method centers about the use of adhesive plaster as a wrapper for well fitted grafts.

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. These studies were begun as a part of the Wisconsin program in the crown-gall project supported cooperatively by the Crop Protection Institute, the Iowa State College of Agriculture and Mechanic Arts, and the University of Wisconsin, and have been continued in cooperation with the United States Department of Agriculture.

[2] While it is outside the scope of this paper, mention should be made at this point of the practice of budding which avoids the kind of union provided by the piece-root graft. The root-knot difficulty has been virtually obviated in many eastern nurseries through this practice. However, for various reasons, most of the nurserymen in the middle west still prefer to propagate their apple trees from piece-root grafts.

The use of adhesive plaster, or nurserymen's tape, has been sufficiently successful in experimental plots to deserve extensive trials by the propagators of apple trees from piece-root grafts. Experiments have been conducted with this wrapper[3] on apple during four years under a variety of conditions covering a geographic range which included Iowa, Kansas, Minnesota, Missouri, Nebraska, Oklahoma, and Wisconsin. The usual practice of employing grafts with a five- or six-inch cion and a three- or four-inch root has been followed. When the plaster was correctly applied to grafts of this size, no trials have yet been made in which it failed to rot before there was danger of girdling. The methods of employing plaster in special cases where a very short cion (two inches long) is used or in regions where irrigation is employed have not been worked out. Under such conditions, it is possible that the plaster might remain too dry and fail to rot soon enough to avoid girdling. While the plaster doubtless has promise of value on a much wider range of plants, results are available only on apple. In the trials made thus far, the plaster, on the average, has shown no influence on the stand of trees when compared with the commercial string wrapper. Considerable fluctuation in stand, both against and in favor of the plaster, has been observed in the same localities in successive years and in different localities in the same year. However, the plaster has shown a distinct influence on the prevention of root-knots at the union. In no single experiment in any year has it failed materially to increase the percentage of trees with smooth unions. The effectiveness of the plaster has varied with the amount of disease present. While less complete control was secured in southern than in northern regions, in some years when root knot was very severe, the value of plaster in the south was emphasized when the percentage of smooth trees wrapped with plaster was compared with that of smooth trees wrapped with the commercial string. A digest of the field data for 1928 on the value of plaster compared with string for the prevention of root-knots has been prepared. This is summarized in table I.

The cost of applying the plaster is comparatively small, and varies with the methods employed. In some nurseries the use of plaster does not increase the cost. On the average, one good tree saved out of a hundred more than pays for the increased cost. No nursery has been found where, when the plaster is correctly applied, the increased cost is not covered by a saving of two good trees out of a hundred.

The method of applying the plaster is quite simple. The grafts were well made in the usual manner from clean cions and roots free from disease.

[3] The nurserymen's tape employed in these tests was supplied by Johnson and Johnson, New Brunswick, N. J. It is a modification of their zinc oxide medical adhesive plaster, the chief differences being the omission of zinc oxide and the use of a cheaper fabric.

TABLE I.—*Summary of experiments in 1928 which compare cotton string and adhesive plaster wrappers in relation to the occurrence of malformations at the union of piece-root apple grafts*

Number and location of trials	Age of trees	Graft	Wrapper	Smooth	Small knot[a]	Large knot[b]
	Years			per cent	per cent	per cent
Average of six trials in Iowa, Kansas, Minnesota, and Oklahoma	1	Wedge	String[c]	59	20	21
	1	do	Plaster	91	4	5
Average of eight trials in Iowa, Kansas, Minnesota, and Oklahoma	1	Tongue	String[d]	56	17	27
	1	do	Plaster	95	3	2
Average of four trials in Minnesota, Nebraska, and Wisconsin	3	Tongue	String[d]	71	5	24
	3	do	Plaster	91	2	7

[a] This class included all enlargements, regardless of cause, smaller in cross measurements than half the diameter of the tree.

[b] This class included all enlargements larger across than half the diameter of the tree.

[c] The stand was reduced in some cases in which the wedge grafts were wrapped with string and subsequently broken in handling. No breaking occurred when the grafts were wrapped with plaster.

[d] The stands of trees wrapped with string and plaster wrappers were approximately the same.

The method was equally successful with either tongue or wedge grafts. A half-inch-wide roll of nurserymen's tape was mounted on a roller at the side of the operator. The tape was applied in a spiral wrap over every part of the union. Care was taken to overlap the edge of the plaster slightly and to make a water-tight covering over every part of the cut surface. The tape was cut to the desired length for the graft in hand just before the final inch was applied. It was cut on a section of a cutter-bar or some other knife conveniently mounted in front of the operator. The tape was applied so that no more than two thicknesses of material circled the graft at any one point. After a little practice 350 to 400 grafts were wrapped in an hour. The grafts were stored and planted in the usual manner.

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PLANT QUARANTINES AND THE STATE

W. A. McCubbin

In looking over the organizations which have been developed in the various States to carry on quarantine and inspection activities, one is struck by the great diversity in the agencies assigned to this duty. It is true that 45 out of 48 States center their legal quarantine power in either a department of agriculture or a State board of agriculture. But these administrative bodies carry out their functions through a great variety of agencies or individual officials. In 4 States, a plant board controls these activities; 23 others concentrate the work in an administrative subdivision, usually a bureau or division devoted to plant industry, inspection, orchards and nurseries, entomology, pest suppression, farms and markets, horticulture and the like. Thirty-two States entrust the quarantine function to an individual who is usually the State entomologist though he may be a chief inspector, supervisor of horticulture, agricultural inspector, State horticulturist, seed analyst, or a general State agricultural officer. In 20 out of 48 cases such official is at the same time on the extension service or experiment station staff. Is this wide diversity in administrative machinery a source of weakness in quarantine enforcement? Does the method of State organization affect the efficiency of quarantine work to such an extent that something should be done about it? Probably not. The administrative function has been demonstrated over and over again to possess such great flexibility that it can exert its full powers through any of the diverse channels mentioned, and one may therefore conclude that almost any type of administrative agency is acceptable, so long as it meets certain basic requirements.

Among these requirements, a well grounded legal authority should undoubtedly be given first place, and this requirement would be closely followed by its complement of adequate funds and staff. Next, the quarantine agency must contain within its own organization or be able to command from elsewhere that technical knowledge which is nowadays in constant demand, not only in forming policies, planning operations, and meeting crises, but in meeting every requirement of ordinary procedure. Again, the quarantine office must be prepared to deal with those phases of its problems which extend into the field of survey, research, service, and public education. Further, provision should be made for close and sensitive contact with all the interests in the State affected in various ways by quarantine activities; and finally the State agency must be equipped in these complex times to maintain itself on a basis of mutual understanding and cooperation

with other States and with the Federal authorities. Granted that these six basic requirements are met, there seems to be no reason why any existing type of State organization should not carry out quarantine functions competently and successfully.

It is not the purpose of this discussion to inquire to what extent individual States fulfill these requirements; but we may properly comment on the relation of these requirements to the general quarantine situation.

The first requirement touching the need for legal power may be passed with the remark that during the last few years there has been widespread tinkering with and remodelling of various State statutes, with the result that many former legal handicaps are rapidly disappearing.

The supply of man and money power is improving somewhat but there is general agreement that it is still far from satisfactory. Not a few of the disappointments and shortcomings in quarantine and inspection work have their origin in the combination of an earnest, conscientious official and a too slender appropriation. All too frequently a sense of public duty urges the planning of a logical program which then cannot be completed for lack of funds.

In this connection there should be mentioned also the numerous cases where precious weeks or months have been lost before the energies of the State could be released by legislative enactment into the field of control. The recent establishment in many States of emergency funds designed to meet sudden outbreaks of insects or diseases indicates that this logical procedure will shortly become a universal policy.

Passing over the need for the best technical knowledge available as a point too obvious to require comment, we come to review the relation of the State quarantine office to certain other closely related activities. Perhaps out of the four mentioned—survey, research, service, and public education—the first is in the most unsatisfactory state. In spite of the lessons of the past, we still continue to impose quarantines without adequate knowledge of the distribution of the pests concerned; yet it is not so much that the quarantine is too hastily applied as that the survey trails too far in the rear. What we need is more and better—and one might add, quicker—surveys.

In the domain of public relations, the quarantine agency has a difficult part to play. It is, on the one hand, dependent on public support and, on the other, subject to public criticism. To develop and maintain the confidence of enlightened public opinion in the State, is important in the present conduct of its work and necessary for its continuity. It is suggested that where any type of regulatory work is established in a State it should be accompanied by a corresponding independent popular body repre-

senting the interests chiefly affected. Through such a body a real and correct understanding of the administrative aims and methods can be widely disseminated and, conversely, popular sentiment on the subject can be gathered and concentrated. A body or association of this kind can furnish support on the one hand and serve as a very efficient check on the other.

Up to this point, the functions of quarantine agencies have been considered only in relation to the State itself. When one comes to envisage regulatory matters from the interstate standpoint, there is at once apparent a somewhat perplexing situation. The perplexity arises partly from the independent and widely varying policies, standards, and procedures extant in the different States, and partly from conflicting opinions as to the respective rôles which should be assumed by the State and Federal authorities in the regulation of interstate affairs.

It is true that the now historic Supreme Court decision of 1925 has helped to clear the air somewhat. Previous to that period, the various States enacted and enforced such quarantines as they pleased almost regardless of Federal quarantines covering the same subject. The Supreme Court ruling held that since the U. S. Constitution vested control over interstate commerce in the Federal administration, the imposition of a quarantine under that authority should imply that due consideration had been given to the matter and that the action taken must be regarded as adequate both as to subject and area. Consequently, all conflicting State action was void. The decision went still further: It took the ground that absence of Federal action should be presumptive evidence that no action was required, and thus, in effect, denied to the States all authority over interstate shipments. If the principle here established were carried to its logical conclusion it would mean Federal control over all interstate shipments of materials concerned in the spread of insects or diseases. Such control must inevitably involve a multiplication of Federal quarantines, vast additions to the Federal staff and enormous increase in Federal expenses.

Fortunately, Congress soon cleared up the situation, providing by resolution an interpretative clause which stated in effect that, until the Federal authority had acted on a subject, individual States were free to take such measures as they deemed necessary. Even with the State power of quarantine thus partially restored there are those who view the outcome of this incident with alarm, seeing in it an encroachment on jealously guarded State's rights. This attitude is perhaps too extreme. With equal justice, the claim could be made that this court decision merely served to restrict the activities of the States within the bounds originally established by the constitution.

As matters now stand, no future conflict is likely to arise from a clash of State and Federal quarantines. But there still remains largely unsolved the original problem of how to obtain a much desired uniformity in quarantine action, inspection, and other regulatory functions among the States, particularly in the field not as yet covered by Federal action.

It is perfectly obvious that further extension into this field of Federal power, backed as it is by constitutional authority, would provide an effective means of unification among the States. But against unlimited extension of this kind are arrayed the economic arguments mentioned, as well as the defenders of State rights.

I am not aware that such extension is desired by any individual, group, or party; one could surmise that the Federal administration grew to its present proportions largely because of a continual thrusting upon it of added duties and responsibilities, rather than because of its grasping disposition. And it is probable that it would be forced into taking control of many matters in the field of interstate relations with great reluctance.

Perhaps the vital objection to placing on the Federal shoulders too great a burden of interstate quarantine and regulatory matters lies not so much in endangering State rights as in encouraging a definite trend away from local self-government. Our form of government depends on the intelligent interest of its citizenship in public affairs and their readiness to step in and help when difficulties arise. With the center of control and responsibility removed to Washington, the nation stands to lose in public knowledge and interest and sympathy and support much of what may be gained in standardization and efficiency. Such action tends to remove from the hands of the people the strings with which they have heretofore been accustomed to control their own destinies.

Still other objections may be raised against certain forms of Federal activity. The difficulties which arise when Federal quarantine action is directed against a small area in the State's interior are well known, and interstate nursery shipments provide an example of the awkward situation which would arise if the Federal power provided by the constitution were exerted in the field of inspection. It is difficult to see how a Federal certificate for interstate traffic could be issued without Federal inspection; yet such inspection would have no jurisdiction over stock grown and sold within the State, itself. Shall we then have two sets of inspectors for the same nursery? That way madness lies.

We are forced to conclude that while the exercise of Federal power to control the interstate spread of insects and diseases is constitutionally unlimited, yet there is a field in interstate relations in which it is subject to practical limitations. In certain problems Federal action will be

entirely beneficial, in others, the benefits will be counterbalanced by definite disadvantages, and in still others the obstacles are to all intents and purposes prohibitive.

It would seem then that we must look elsewhere for help in straightening out the tangles in certain aspects of interstate relations. To what source may we turn? At the present time the most hopeful outlook lies in agreements made between the states themselves. If these political units, recognizing the difficulties of the situation, can get together on matters of general policy, agree on inspection standards, provide for uniformity in procedure, and plan for concerted action in a common cause, the most pressing of their problems can soon be solved. And to facilitate just such action, we now have the Plant Board system.

The organization of the four regional boards composing the plant-board system was modelled largely on that of the Western Plant Quarantine Board, and it was the success of this body in the Pacific Coast States which suggested the formation of similar systems in three other regions. The States comprising each of these regions have many common problems arising out of geographic and climatic conditions and similarity of crops. The groupings thus formed outside the Western Board include the Eastern Plant Board covering the New England and Middle Atlantic States, the Southern Plant Board, composed of the States in the Cotton Belt, and the Central Plant Board embracing the great cereal district of the upper Mississippi basin.

In each State within a district, the officials in charge of quarantine and regulatory work appoint representatives on the regional board, usually for yearly periods. The assembled board elects its own officers, adopts its own constitution and by-laws, and, in short, conducts its affairs as an ordinary conference body. It is understood that the relationship of these boards to the States in their region is of a purely advisory nature and that no State administration is bound by decisions of the board or by commitment of members.

When the formation of these four boards was completed, every State in the Union was represented, and the next step was to provide a means whereby the work of the regional boards could be nationally correlated. For this purpose, each regional board was asked to appoint two representatives, the eight thus chosen to form the National Plant Board. This body was intended to function in a national way in much the same capacity as the regional boards in their groups of States.

What are the aims and functions of the Plant Board System? In the first place, the most urgent responsibility before these boards is an endeavor to bring about in each regional group and even on a country-wide basis, as

far as possible, uniformity among the States in the field of inspection. The nursery inspection service is in dire need of such reformation and is being given much attention. It is realized that the unification process must reach deeper than the mere form of certificates used and must cover such things as standards and even methods of inspection.

Second, the plant boards recognize the value of agreeing on a common policy in the establishment of interstate quarantines. It may be noted here that the National Plant Board is now attempting to draw up a set of principles of quarantine which it is hoped may serve as a guide in quarantine procedure.

Third, these bodies serve as a valuable clearing house for the exchange of ideas on general policies and practices in disease and insect control.

Fourth, the plant-board system provides, on the one hand, an orderly and organized channel through which the collective opinions, suggestions, and requests of the various States can be presented to the Federal authorities, and on the other hand, it represents a widespread influence which may be needed from time to time in the support of Federal undertakings.

From this brief outline of organization and purpose, it may be gathered that the plant board system, while no doubt conferring much benefit on each State because of the constant interchange of ideas on methods and policies, has as its great field of usefulness those matters which concern interstate as well as Federal-State relations. And since this field just now presents many perplexing problems, there exists a very excellent opportunity for this new type of organization to distinguish itself.

If the plant-board system can help in the solution of these interstate difficulties it may not only become a valuable and permanent acquisition from the State's point of view, but may be welcomed by the country at large as a hopeful means of settling many awkward features of Federal-State relations.

BUREAU OF PLANT INDUSTRY,
HARRISBURG, PENNSYLVANIA.

SOME PRESENT-DAY PROBLEMS[1]

H. P. Barss

In the past twenty years which have elapsed since the founding of the American Phytopathological Society there have been great developments in our knowledge of plant diseases and in our methods of dealing with them. The contributions to this progress which have been made by our members may well be a source of gratification to the founders of this Society. Throughout these two decades this organization has fostered a spirit of comradeship and exerted a stimulus to achievement the effects of which cannot be underestimated.

It would be interesting to look back over these years and survey the advances which have been made; but, in discharging the responsibilities of the office with which you have honored me, I would prefer rather to look forward and discuss with you some of the problems which challenge our attention as plant pathologists and members of this Society.

Phytopathology. Of most direct concern, undoubtedly, to every member of this Society is the problem of how to insure adequate present and future support for our journal, Phytopathology. Our Society was launched partly to meet the need for such a journal. The journal has grown as the Society has grown but in recent years the great increase in research into plant diseases has tended to bring to it longer articles dealing with more detailed data than formerly. Publication costs have therefore grown out of proportion to the income. We are fortunate in the able editors and competent business managers we have had to direct the progress of the journal, but it is perfectly plain that the best efforts of those in charge cannot any longer enable Phytopathology to meet the requirements of the membership and at the same time continue on the present financial basis.

In this situation, the natural and obvious course is for us to adopt the recommendations of the Council and increase the membership dues, allowing the added sum to go to the journal. This seems a very reasonable proposal and to my mind deserves unanimous support because it is apparently the simplest and most direct way, partially to meet the need.

In addition to the proposal for increase in the annual dues, there have come other suggestions which deserve thoughtful attention. There is strong belief among our members that the future security of our journal would be best assured if a permanent reserve fund could be gradually built up. The interest from this reserve fund could be used to meet the special

[1] Presented as a presidential address at the Twentieth Anniversary Meeting of the American Phytopathological Society at New York City, December 28, 1928.

needs of the journal and there would be greater financial stability. With this in mind the creation of an endowment fund for PHYTOPATHOLOGY has been proposed as a memorial to the late beloved George R. Lyman. The idea is fine. A drive for an increased number of life-memberships has also met with considerable favor. Some there are who would seek outside support, but it is a mistake, I think, for us to hope for any important contributions from outside our ranks. The men of wealth who are assisting other branches of science have had, for the most part, no contact with those problems of agriculture and forestry with which it is our responsibility to deal. Lack of contact means lack of understanding and hence lack of real interest in what we are striving for. We must count on nobody but ourselves. Let us put our shoulders to the wheel, then, and push this vehicle of science, PHYTOPATHOLOGY, over onto the smooth and solid highway of financial security.

Biological Abstracts. There is another problem from which we cannot escape. That is the problem created by the enormously rapid growth of scientific literature due to the constantly increasing volume of research. We all realize that the only practical way of meeting this situation lies in the development of a comprehensive abstracting and indexing service. *Biological Abstracts* has been launched by the Union of American Biological Societies to take care of this great need. It was a courageous and difficult undertaking. The Editor in Chief, with the assistance of his able staff and the help of a host of voluntary collaborators, has labored to develop a sound foundation for this essential enterprise. These workers have been confronted by most complex and sometimes disheartening problems, but they have stuck doggedly to the work and deserve our fullest commendation and support. To insure the permanent success of the undertaking, however, more adequate capital and a larger income are imperative. Serious consideration should be given by this as well as all other interested societies to every proposal that aims to bring these to pass.

It would help materially if, for one thing, all members of biological societies, including our own, were to subscribe individually for *Biological Abstracts.* Under the circumstances, I am inclined to look upon this as almost a professional obligation. Yet there is good reason to doubt if increased subscriptions alone will ever be able to solve the whole problem. I am compelled to believe that a large permanent endowment is necessary if *Biological Abstracts* is to operate on a basis satisfactory to the sciences which it serves. Every biological society, ours included, should lend active moral support to any appeal for endowment which is made.

In case adequate endowment cannot be obtained, why should not those departments of our universities, colleges, experiment stations, Government

offices, etc., which have to do with any branch of biology, become voluntary supporting agencies by placing in their annual budgets a definite item for *Biological Abstracts?* It would need to be but a very small percentage of the total budget. At first thought this may appear highly visionary and impracticable but it was just such voluntary support by interested educational institutions which first insured permanency to that world-famous scientific institution, the Woods Hole Marine Biological Laboratory.

If all other plans should fail, I would not look upon it as out of order to suggest that the Federal Government undertake to subsidize *Biological Abstracts* as a direct aid to the scientific work of the Nation, in which the scientific bureaus of the Government will be among the most important beneficiaries. The greatest objection to this course is that the truly international character of the undertaking would be jeopardized.

The important thing is to find some way to insure sufficient income to free *Biological Abstracts* from its present financial restrictions, to render it less dependent on voluntary assistance for the bulk of its abstracting and in this way to greatly facilitate promptness as well as completeness in publication. I suggest the appointment of a committee by this Society to further the interests of *Biological Abstracts* and to keep us in touch with the situation.

Plant Quarantine Procedure. Let us swerve sharply away from these financial questions to the kind of problem that is presented when a dangerous plant disease is first found invading new territory. We have just listened to a discussion of how the Federal Government and the States are handling such problems. This is a subject so important that it deserves the active interest of every plant pathologist. I pass on to you the query of one of our membership of long experience. "Is it not likely that future phytopathological historians, in looking back over the twenty years just ahead of us, will have reason to characterize the period as one chiefly distinguished by the discovery of and attack upon a succession of serious introduced plant diseases?" Let us give this a moment's contemplation. Now let us ask ourselves whether, even with our past experiences, we are really prepared to deal with new plant pest invasions and extensions in an intelligent and effective manner.

Would it not be an advantage if there existed a carefully considered and generally accepted course of procedure for dealing with such invasions at the very outset? Delay is costly when it comes to stamping out or holding in check such incursions; but hasty action on the basis of inaccurate or incomplete information has resulted frequently in much fruitless effort and expense. What is wanted is a wise and logical program, advancing by well coordinated steps, which could be put into effective operation in most cases just as soon as a dangerous new disease is brought to light.

One thing that strikes us immediately in regard to our present situation is the common lack of adequate provision for emergency funds and authority to deal promptly with such outbreaks. Furthermore, present procedure varies greatly. In some quarters, there seems to exist a tendency to try to cover any situation by quarantine orders before adequate surveys and studies have been conducted; while in others there is a tendency to delay all protective action until complete information is available. The evil consequences of hysteria on the one hand, and inertia on the other, can be avoided only by intelligent planning and preparation.

To aid in preventing unnatural spread of newly introduced plant pests, plant quarantines are now quite generally employed. Is there not urgent need for a statesman-like analysis and codification of the principles on which they are based? Furthermore, the general public seems to feel that there is confusing diversity of wording and complexity in the provisions of both general and local quarantine orders which might be replaced by simplification and unification along well-thought-out lines. I wish to encourage active cooperation in the improvements in these directions which are being fostered by Federal and State authorities and by the regional and national plant boards.

As we all know, there are a strong current of opposition to plant quarantines and a tendency on the part of affected commercial interests to attack individual quarantines. If every quarantine order were based on certain established and well-defined principles embraced in an accepted code, it would be much more difficult than it has been for opponents to attack individual orders. It would be necessary for them in any particular case to challenge either the assumed facts which led to the order or the particular principles on which its provisions were based. I feel that a code of jurisprudence, if you please, pertaining to plant protection must come, in time, as a guide in the development of sane, sound, and effective measures of plant defense over the whole world.

Do not such problems deserve more attention from our Society than has been given in recent years? Would it not be worth while to have at least a standing committee on plant quarantines and regulatory work to keep the members in touch with progress made each year along these lines?

Right here, I would like to express my own hearty assent to an idea set forth by our own first president, Dr. L. R. Jones, in an address before the International Congress of Plant Science at Ithaca in 1926. It was a suggestion that no plant-quarantine measure should be adopted without the appropriation at the same time of funds for investigations, the object of which would be to find a way to render such quarantine ultimately unnecessary. I believe that this plan is fundamentally sound. I think it could

and should be applied in the great majority of instances, at least, where internal quarantines are invoked.

Foreign-Pest Investigations. A year ago, this Society passed a resolution advocating extension of the study of dangerous foreign pests in their native environment. The committee appointed to represent us has reported on its initial activities. The importance of foreign investigations looms up larger than ever. To illustrate, we now realize the menace to our great western forests of Douglas fir, yellow pine, and Sitka spruce created by the advent of the European larch canker to this continent. This disease should be more thoroughly studied at once and Europe is the only logical place for this. Again, America is much concerned over the evil potentialities of the European elm disease. We are glad that the cause has been established beyond doubt, but we would feel less apprehensive over possible introduction, if practical control measures could be worked out through investigation. Such investigation can be prosecuted, of course, only beyond the Atlantic. I suggest that the Society continue this committee with an expression of our appreciation of its efforts and support of its aims.

Research on Fungicides. Let us turn now to a phase of plant pathology in which our scientific progress seems to have lagged far behind that which has been achieved in other directions. I refer to our knowledge of fungicidal materials, fungicidal action, and the relations which the state of the host, state of the pathogen and state of the environment bear to the effectiveness of fungicides on the one hand and to the presence or absence of host injury on the other.

We owe much to the handful of able investigators who are now digging patiently beneath the surface in this most important field, but the task is so great and the advance is necessarily so difficult and slow that I would like to send out a call for reinforcements.

When we squarely face the situation, we are forced to admit that in this age of chemical advancement, we, as plant pathologists, are still almost entirely dependent on the same old half-dozen or dozen chemicals or chemical combinations we were using as protective and disinfecting agents at the time this Society was founded. What is worse, even in respect to these old and familiar materials, we still know but little about their modes of action or their range of effectiveness or safety under diverse conditions. I think it would be unjust to intimate that censure is due the profession because these things are so, but I feel that we shall certainly be called to account if this state of affairs is allowed to continue any longer.

Our need for fundamental information is very real. For instance, as a guide in developing control programs for new diseases or for old diseases in new situations, we ought to have a series of monographic studies of our

chief present-day fungicides and the conditions affecting their action. Then, too, there should be some way provided whereby the new types of fungicides brought out from time to time are promptly subjected to standard tests to determine their range of applicability and their limitations. Surely we ought, also, to be probing into new chemical channels for possibilities that we can make use of in better plant-disease control. At any rate, the time seems to have arrived when sporadic, spasmodic, desultory, and purely empirical investigations on fungicides ought to be supplanted, or at least supplemented, by more adequately financed, more adequately manned, more methodical, more comprehensive, and more prolonged researches in this important field.

Because of the nature of the problems involved, research in fungicides can make the most consistent and rapid progress only where groups of adequately trained investigators, provided with suitable laboratory, greenhouse, and field equipment, can devote themselves exclusively to this type of work over long periods of years and where they can command the cooperation of skilled chemists, of plant physiologists, and of field workers acquainted with the diseases of various crops. There probably are very few of our State experiment stations that could meet these essential requirements in an entirely satisfactory manner because of limitations which cannot be overcome. The United States Department of Agriculture, however, can provide exactly the necessary conditions for such a program if furnished the necessary funds. An undertaking of this sort would be consonant with the purpose for which the Department of Agriculture was established, namely, the solution of problems affecting the agricultural prosperity of the whole Nation. I believe that the time has come when this Society should urge the Federal Government to provide greatly increased funds for use by the Department of Agriculture for fundamental research in fungicides. If this is to be accomplished, our members must help to secure strong support from all the States for the necessary Congressional appropriations.

Expansion of Cooperation. Everybody is agreed that cooperation makes possible accomplishments which could never be attained without it. There is evidence of a very rapid increase in cooperative attack on individual plant-disease problems in recent years. I believe we are warranted in encouraging still greater extension of the principles of cooperation in the field of plant pathology but success will not follow such efforts unless at the same time we do all we can to remove the stumbling blocks which always beset the highway of cooperative progress. Most of these stumbling blocks, if turned over, will be found to have on the bottom the label "selfishness" or the label "misunderstanding."

There are different sorts of cooperation, all of which are useful, but I wish to speak particularly in favor of the cooperative relations which are growing up more and more between the offices of the Bureau of Plant Industry and the plant pathology departments of the various State experiment stations. Here is a realm of cooperation in which, when successful, the Federal office, the experiment station, and the grower all benefit. Federal cooperating workers, tackling problems from a wide angle, from more than a local viewpoint, are not restricted in their movements by State boundaries. They also have fullest access to the great scientific resources of the Washington organization and are able to draw directly on the breadth of experience and the knowledge possessed by its personnel. When headquartered with one of the experiment stations, a Federal investigator usually is provided not only with required office, laboratory, greenhouse, and farm facilities but he has direct access to the advice and counsel of the local investigators and their colleagues based on their knowledge of local conditions and their practical experience with the problems in hand which cannot be secured in any other way. Furthermore, the experiment station is able to provide means of connection between the Federal workers and growers possessing the right qualifications for cooperators in field tests, etc. In addition, the interested experiment stations are in a position to see that public support is maintained in behalf of the Federal appropriations required to conduct such cooperative projects. There are many States which recognize the tremendous value to themselves of such cooperation and there is a growing realization by the Federal offices that much more can usually be accomplished per dollar expended where travel and subsistence, as well as laboratory and other costs, are reduced by having an investigator placed at an experiment station in the region where the problem to be studied exists.

Besides such Federal-State cooperation, I wish to call attention to the growing tendency toward cooperation on individual projects between different departments in the same institution. This sort of cooperation has its difficulties, of course, but the effort to surmount these difficulties is more than compensated for by the fact that it makes possible results that would otherwise be impossible. We already have examples of such collaboration between departments of plant pathology, on the one hand, and departments of genetics, plant physiology, soils, chemistry, entomology, bacteriology, agronomy, horticulture, etc., on the other. It is folly for a plant pathologist to attempt to become a specialist in every field of knowledge which must contribute to the practical solution of an economic plant-disease problem. It simply cannot be done. The only alternative lies in the type of cooperation just mentioned. I believe in the encouragement and utiliza-

tion of individual ability and individual initiative in science, as well as outside; but I believe that the greatest advances can be made only where the wisely coordinated efforts of various individual specialists are focussed effectively on one and the same problem.

Transportation and Market Pathology. My time and yours is slipping away but I would like to touch briefly on a few other topics. In its final analysis, our work as plant pathologists, has as its basic purpose the destruction of waste—waste due to diseases of plants and decay of plant products. Much progress has been made in recent years in reducing that form of waste which is due to decay in perishable products in transportation, storage, and handling. The losses from deterioration of crops after they leave the hands of the farmer, however, still continue to be far too large. It is fitting that on our program time has been set apart for special consideration of the problems of transportation and marketing pathology. We are also fortunate in the opportunity which is to be ours to observe first-hand some of the wonderful facilities which New York City provides for handling perishable products with a minimum of loss from deterioration.

Disease-free Seed and Planting Stock. I rejoice to see the rapid strides we are making at the present time in another important direction. I refer to the efforts which have been begun to put into the hands of the farmer and fruit grower seed and planting stock that will not introduce into his field the plant diseases that have hitherto everlastingly eaten into his hard-earned income. Every bit of progress in the certification of disease-free stocks attested to by trained and impartial inspectors, every forward step in the chemical and thermal treatment of seeds, bulbs, and nursery stock, and every advance in the propagation of disease-resisting varieties deserve the fullest support of our profession and of the public. We must not underestimate the difficulties involved in applying this principle generally. The path is not nearly so smooth as it looks from a distance and progress will not be so rapid as we might hope, but I believe that no tactics against plant diseases possess a sounder basis.

Graduate Fellowships. May I turn your attention for a moment to another subject. Only the development of the graduate fellowship system has made possible the rapid advance which plant pathology has achieved in the last decade. Through this system most of our younger plant pathologists, without independent means, have been able to complete their advanced training. The system, however, can be carried beyond a safe limit, and we must be watchful that this limit is not overstepped. I refer to the time when by the aid of fellowships we might prepare more well-trained investigators than there are full-time positions to send them into. We must see to it that a proper balance be maintained between the number of graduate

students and the number of full-time openings annually available. Otherwise, we may find America in the situation which is said to have existed in some branches of science in Europe before the war when eager competition was alleged to exist among young doctors of philosophy for every available $600-assistantship.

The Amateur Plant Pathologist. May I interject here something irrelevant? I want to say it and I don't know where else to put it. In the United States, at the present time, many young men of scholarly rather than commercial instincts are now inheritors of wealth. For such the sciences can provide pathways to lives full of interest and usefulness. Few branches of science can offer fuller satisfaction or a greater sense of worthwhile accomplishment than that of plant pathology. The opportunities which our larger institutions, particularly, have to interest young men of independent means to enter the field of plant pathology should not be overlooked. We need more workers who can devote their lives to pathological studies without the limitations imposed by public demands, by restricted funds, by project outlines, and by administrative duties.

In closing, may I predict that as civilization marches forward the work of the plant pathologist will grow constantly more important as well as more interesting. Science is engaged in a great campaign for the advancement of human welfare through the conquest of nature. Plant pathology holds an important position along the battle front. The world has entrusted to us the practical control of its plant-disease difficulties. Our responsibility is large and, in the discharge of this responsibility, many problems are bound to confront our profession. It is my hope that every member of this Society will take an active part in working out their solution.

OREGON STATE COLLEGE,
CORVALLIS, OREGON.

THE SECOND DECADE OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY[1]

R. J. HASKELL

Twenty years have elapsed since a small group of plant pathologists, feeling the need of an organization for the promotion of their science, conceived the idea and laid the foundations for this organization, in which we are all proud to claim membership, the American Phytopathological Society. That their belief in its cause and hopes for its success were fully justified is evidenced by this large gathering of members here to-night. We are happy to honor and to congratulate the charter members and officers on this occasion.

The history of the first decade of this Society was very ably outlined by Dr. Shear on the occasion of the 10th anniversary meeting in Baltimore. It was his suggestion at that time that the Secretary should prepare a brief historic sketch once each decade. Since this duty and privilege fall upon me, I shall very briefly mention some of our more outstanding accomplishments since 1919, and at the same time indicate a few paths along which we might with profit direct our steps in the future. I am somewhat handicapped, however, by the restriction of my time to ten minutes, thus making it necessary to review these events at the rate of one year per minute.

The growth in membership of an organization is an indication of its success. This Society started with one hundred and thirty charter members. Within ten years it had trebled its membership and to-day it is about six times its original size with 794 members.

During recent years an increase in the number of members in other countries has been very noticeable. In 1918 there were fourteen members living outside the United States and Canada. To-day there are eighty-five such members. As far as I know no special effort has been made to solicit these memberships. This indicates that our sphere of influence is extending and that foreign lands may become increasingly important sources of new members in the future.

Another indication of growth is the attendance and number of papers presented at our annual meetings. Whereas, the average attendance of members during the first decade was fifty-six, that of the second decade was one hundred and thirty-six (exclusive of 1928). The average number of papers presented per meeting during the first decade was thirty-eight, while the corresponding figure for the second ten-year period was practically

[1] Read at the Twentieth Anniversary Meeting of the Society, New York City, December 29, 1928.

double with seventy-nine. This has led to congested programs and annual discussions concerning the limitation of papers and the editing of abstracts. The method of simultaneous sessions recently followed has helped to relieve the congestion of the program. However, there still is opportunity for improvement in the general quality of the papers submitted. Could the standard possibly be raised by offering a prize or by giving honorable mention for the best contributions to the program? Is there any reason why we should be among the last of the several scientific organizations to establish prizes or medals for the stimulation and encouragement of high-class research, these awards to be given for the best publication of the year or for the best paper on the annual program?

I might point out at some length the great growth and development that have taken place in our Journal, but for lack of time I will simply say that PHYTOPATHOLOGY is now the leading publication for this science in the world. We are justly proud of it, and we wish to take this opportunity to thank again the various editors that have brought PHYTOPATHOLOGY to its present high state of development.

In looking over the past reports of annual meetings one cannot but be impressed by the work of the Advisory Board. Since its establishment exactly 10 years ago it has set its mark high up on the wall of outstanding and unusual accomplishment. One of its important functions has been the arranging and conducting of summer meetings once each year. These summer meetings have all been successful and have come to be looked upon as an established activity. I do not need to take the time even to outline the activities of the Board. These are a matter of record.

The Society is to be congratulated on the gifts and contributions that it has made to worthy causes. The members have ever been ready and willing to pass the hat when occasion demanded. To the Oberly Memorial Fund, for encouraging the compilation of bibliographies in the field of agriculture and the natural sciences, we contributed $100.49. For the Cuboni Memorial in Rome we sent $25.00 in 1922. Towards the wiping out of the deficit on PHYTOPATHOLOGY in 1920 and 1921 we made individual voluntary contributions amounting to $1,059.72. For the use of the International Congress of Plant Sciences at Ithaca we gave $200. To European phytopathologists stricken by the calamity of war we sent back-volumes and numbers of our Journal, free of charge, and since that time have given them special subscription rates.

To give to these worthy enterprises is a good practice and one that should be continued. The opportunity presents itself at this meeting to make a contribution of this sort with a two-fold purpose in mind, (1) the establishment of a memorial to a past Secretary and President of this Society

who gave unhesitatingly of his time and energy for its welfare, and (2) the establishment of a greatly needed endowment fund for PHYTOPATHOLOGY.

Two projects which have become realities during the period of this report are *Phytopathological Classics* and the Pure Culture Supply Laboratory. Mention also should be made of the important work on crown gall now going on largely because of the impetus given the subject at our Cincinnati meeting.

Any review of our history would be incomplete without mention of our Canadian and Southern Divisions which are celebrating their 10th anniversaries this year. Affiliated with the Society in 1918 they, like the earlier Western Division, have steadily progressed and thoroughly demonstrated their usefulness. We are proud of our three Divisions and extend to them our best wishes and support. The time should not be far distant when other similar branches, such as a Philippine or an Hawaiian Division, will be organized. Our name is broad enough to include any country, or group of countries, in the Western Hemisphere.

These suggestions naturally lead to thoughts of international organizations. There is need for an active, permanent, international association of plant pathologists. The International Institute of Agriculture at Rome does not seem to offer much hope. The Pacific Science Congress which meets every three years has sessions at which papers on plant pathology can be read. The International Committee of Phytopathology and Economic Entomology has been working diligently on this matter since the successful Holland Conference in 1923. It has attempted to arrange for other conferences and has made several proposals for an organization, chief among which is what might be termed an "International Association of Institutions for Plant Protection." Although this may be desirable, it does not, at the present time, seem to be entirely practical from the standpoint of our science. It would appear to be more for the benefit of persons engaged in plant quarantine and inspection than for those engaged in research, teaching, or extension work in plant pathology or entomology.

Another and probably the most important international body for us is the International Congress of Plant Sciences. The Section of Mycology and Plant Pathology is organized and officered temporarily by workers of the country acting as host. Would it not be desirable, as well as practicable, to organize this Section more permanently? An International Federation of Plant Pathologists and Mycologists made up of representatives of the Societies in the various countries could assist in coordinating one Congress with the next; plan new proposals to come before the Congress; assist the Pacific Science Congress in arranging its programs; arrange for other international meetings; encourage important researches and

surveys; facilitate the preparation and publication of compilations, monographs, indexes, etc.; and otherwise promote the science generally.

During the past decade, advances in plant pathology have been exceedingly rapid. Ten years ago, research on the virus diseases was in its infancy; copper carbonate for treating wheat to prevent bunt was unknown in this country; organic mercury compounds and several other seed and soil disinfectants had not been heard of; the Plant Disease Survey was just getting started; extension pathologists were employed in only two States; and numerous other illustrations might be cited. When one stops to consider these facts, he cannot but be impressed by the progress that has been made. Our Society has kept pace with these advances. It can be predicted with certainty that it will continue to do so, and will become an ever increasing influence for the promotion of plant pathology throughout the world.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

PHYTOPATHOLOGICAL NOTES

Black-stem disease of alfalfa, sweet clover, and red clover.[1] During a study of leguminous forage-crop failures in Kentucky, covering the past seven years, the writers have had under observation a little-known disease of alfalfa, sweet clover, and red clover which causes blackening of large areas of the stem, and suggests the name black-stem disease. The lesions on sweet clover are particularly prominent, often blackening the entire base of all the plant shoots. The lesions are usually smooth and black and, according to our observations, fruiting bodies are not produced during the current season. The disease on alfalfa is probably often confused with Sackett's bacterial disease. The true cause of black stem is not definitely known, but is being studied. It is probably of fungous origin.

Under certain conditions, the disease apparently has caused serious damage to each of the crops mentioned. Observations in 1921 and subsequently, on thick stands of second season sweet clover subjected to various clipping practices, gave the first indication of the seriousness of the disease. Plants extensively blackened on the lower portions of the stems died if cut back to the blackened area, as the buds in the blackened zone appeared to have been killed. However, if the stems were free from this disease, buds would develop into new branches following cutting of the stem above them. In 1927, the spring season was very early and wet in Kentucky and black stem was severe on sweet clover and alfalfa, killing many shoots of the latter and apparently causing death of plants in thick stands of the former. In the spring of 1928, alfalfa fields in northern Kentucky were reported to have been severely injured during the winter, many plants in fields over two years old being killed.[2] An examination of these plants showed that the crowns were dead or dying even though the roots immediately below were healthy and well filled with starch. All stages were found, from plants with crowns completely killed to plants with many of the crown buds alive. The injury to the crowns seemed to be of the same nature as the black stem trouble prevalent the previous spring. The fall of 1927 was mild and late, probably giving excellent conditions for the development of the disease until late in the fall. Perhaps fall infection of crown shoots may have accounted for their death during the winter and early spring. The injured shoots, usually about 1 to 2 inches long, were killed back completely, or the tips were killed, in which case lateral growth developed. Often, when one of these lateral branches from the base came in contact with

[1] Printed with the permission of the Director of the Ky. Agricultural Experiment Station.

[2] Many plants in wet-soil areas had heaved but these were readily distinguished from the crown-injured plants.

the dead tip, the black-stem lesions developed on the new growth at the point of contact.

In the problem of red-clover failure, the disease appears to be important as a cause of differences noted between second-season stands of adapted and unadapted lots of red clover grown at Lexington. In the spring of 1927, the stand of year-old European and American clovers[3] which started into growth varied considerably, but all, except the Italian, had a sufficient stand for a good crop. Later in the spring, marked differences in rate of growth occurred and, at blooming time, the foreign strains had but few blooms, while the best American strains bloomed profusely. A close examination at this time (June 10, 1927) showed that the first-crop shoots of French clover were practically all dead and that their present stem growth consisted of second-crop shoots. In the better American clovers with larger amounts of bloom, the degree of blooming was inversely proportional to the amount of death of the first-crop shoots. The stand of plants which remained after first cutting was directly proportional to the number of plants which retained their first-crop shoots until harvest. In other words, those lots, the hay crop of which consisted largely of second-crop shoots, died out nearly completely after first cutting, whereas those lots which bloomed most profusely and had the smallest number of second-crop shoots cut with the first crop had the highest survival after first cutting. The black-stem disease appeared to be the immediate cause of death of the first-crop shoots, although other predisposing factors, as leaf-hopper injury the previous season, may have played a part.

Whether or not black stem is concerned in winter killing of red clovers cannot be stated. It may be of significance, however, that the extent of winter killing during two winters has been in direct proportion to the injury caused to the spring growth of the same types of clovers in the spring of 1927, apparently by the black-stem disease, and this was directly proportional to death of clover plants following first cutting.

The disease develops during the spring or early summer, affecting the first cutting of alfalfa, but largely disappears during the remainder of the season and during a dry spring such as the present one (1928). On sweet clover, it probably is unimportant except in regions where the springs are long and likely to be damp. Then it causes damage only in thick stands or where grass and weeds are abundant or when the clover is clipped with the object of obtaining both a hay and seed crop. It appears to play an important part in thinning out red-clover stands the second year, especially

[3] These clovers consisted of various lots and strains of European and American origin which are being tested cooperatively with Dr. A. J. Pieters, Bureau of Plant Industry, U. S. Department of Agriculture.

in unadapted strains.—W. D. VALLEAU and E. N. FERGUS, University of Kentucky, Lexington, Ky.

A disease of Lippia caused by Sclerotium rolfsii Sacc. Sclerotium rolfsii Sacc. is known to attack a wide range of host plants. It has been found several times previously in California. However, to the writer's knowledge, its occurrence on this particular host has not been previously recorded.

Lippia canescens H. B. K. is a widely spreading, canescent, herbaceous perennial belonging to the Verbenaceae. The creeping stems become somewhat woody at the base, and form roots extensively at the nodes. The numerous upright branches arising from these stems produce a dense green turf. The plant is well adapted to light sandy soils that are moist or can be kept so by irrigation. In the interior valleys of southern California, it often is used as a lawn plant.

In August, 1927, the writer's attention was called to a disease affecting the Lippia lawn of the U. S. Department of Agriculture Cotton Field Station at Shafter, California. It had been noted for the first time during the summer of the previous year when a single, small, affected area was observed. In June, 1927, however, many new and scattered diseased areas began to appear. These increased in size rapidly during the hot summer months, causing large dead spots in the lawn.

The first symptoms appear in early June when the daily temperatures become favorable for the rapid growth of the fungus. A small, greenish yellow spot about a foot in diameter appears in distinct contrast to the green of the rest of the lawn. On close inspection, an abundance of white fungus mycelium is found on the creeping stems of affected plants and on the shaded surface of the adjacent moist soil. The tender, green parts of the plants are soon killed and when dry, form a fairly well defined, grayish white, dead spot in the lawn. The actively growing fungus mycelium at the margin of this spot attacks the healthy plants beyond and continues thus to enlarge until fall. As the protective ground cover is removed and the soil dries out, the mycelium within the diseased area forms numerous sclerotia and becomes inactive. At the center, some of the woody stems which had not been killed, resume growth (Fig. 1). As the Lippia continues to grow during the winter and spring, when the temperature conditions are unfavorable for the active growth of the parasite, the area becomes overgrown by spring, leaving no trace of the spot. Later, when conditions are again favorable, the sclerotia germinate, producing new infected areas, as was the case in 1928.

For the purpose of isolating the causal organism, a block of turf cut from the margin of a spot was brought to the laboratory, placed in a moist

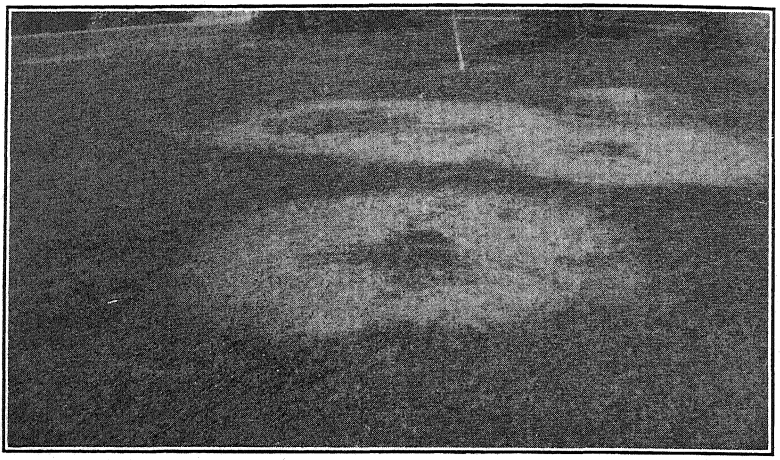


FIG. 1.—Diseased areas in the Lippia lawn of the U. S. Cotton Field Station, Schafter, California. Note the plants which have resumed growth in the center of the larger areas. Picture taken October 16, 1927.

chamber, and held at room temperature. Within a few days, a vigorous mycelial growth covered the entire block. Transfers made to glucose-potato-agar slants developed as pure cultures of *Sclerotium rolfsii* Sacc. producing sclerotia abundantly. Numerous sclerotia of this fungus were also obtained from the block of infected turf.

Pure cultures grown on glucose-potato-agar plates at 30° C. for 4 days show an abundant radial growth of white mycelium. The hyphae branch freely and contiguous hyphae frequently anastomose, forming strands. Clamp connections are common in the main hyphae. Numerous, small, spherical, dark brown sclerotia soon form.

Measurements of 100 sclerotia taken from agar plates showed a variation in size of from .84 to 1.75 mm. in diameter, the average diameter being 1.09 mm. The same number of sclerotia taken from the block of infected turf varied in size from .94 to 2.01 mm. in diameter, the average diameter being 1.29 mm.

The pathogenicity of the fungus was established by successful inoculation of healthy plants grown in the greenhouse.—P. A. MILLER, University of California, Citrus Experiment Station, Riverside, California.

REPORT OF THE TWENTIETH ANNIVERSARY MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE NEW YORK MEETING

The twentieth anniversary meeting of the American Phytopathological Society was held in New York City, December 28–31, 1928. The meetings were held at Teachers College, Columbia University, and the hotel headquarters were the Hotel Lincoln. About 200 members were in attendance.

There were 107 papers on the program, 9 in general session, 4 in joint session with Section G, 11 in joint session with the Mycological Section of the Botanical Society of America, 4 at a session on plant quarantines, 4 by business men on the relation of plant pathology to distribution and marketing problems, the president's address, and the remainder in sessions on cereal diseases, 18, vegetable diseases, 14, fruit diseases, 11, bacterial diseases, 12, diseases of ornamental plants, 10, and miscellaneous crops and diseases, 9.

At the first session of the Society, December 28, the subject of plant quarantines was considered. W. A. McCubbin presented a paper on "Plant Quarantines and the State"; C. L. Marlatt spoke on the "Federal Plant Quarantine and Control Work Reorganized", and C. R. Orton presented the report of the Committee on Foreign Plant Disease and Pest Investigations. Discussion was followed by a stimulating address by the President, H. P. Barss, entitled "Some Present-day Problems" (printed in this issue).

Two papers on the program of the joint session with Section G were of especial interest to pathologists—(1) "Heterothallism in the Rust Fungi" by J. H. Craigie, and (2) "Recent Studies of Curly Top" by M. B. McKay.

The production and distribution of disease-free seeds and plants were discussed at the conference on extension work. Beans, tomatoes, cabbage, and sweet potatoes were considered especially and committees were appointed to prepare summaries of the available information on supplies of disease-free seeds and plants of these four crops.

At the supper and smoker, Sunday evening, December 30, the general subject of market pathology was discussed and short talks were made by J. H. Butler representing the American Railway Express Company; A. U. Chaney for the distributors of fruits and vegetables; Frank Horne for the cold-storage interests, and H. G. Taylor for the railroads. The next morning, starting at four o'clock from the hotel, about 80 persons took the motor coach trip to the Pennsylvania Railroad terminal piers, to one of the Merchants Refrigerating Company's warehouses, and to the Washington Street fruit and vegetable wholesale houses. This trip gave those who took it a very good idea of how produce is handled in New York. It was very apparent that high-quality, well-packed and disease-free fruits and vegetables have a great advantage over inferior products on the New York market.

The twentieth anniversary dinner was held Saturday evening, December 29, at the Hotel Astor and was attended by 186 people. Charter members of the Society were identified by appropriate badges and, when asked to stand, made an impressive group. At the head table were seated ten former presidents, each of whom made brief remarks when called upon by President Barss. Dr. J. C. Arthur, the oldest charter member, speaking briefly and to the point, was heartily applauded. The Secretary-Treasurer read a short report entitled "The Second Decade of the American Phytopathological Society" (printed in full in this number of PHYTOPATHOLOGY). Following the speaking, alleged newspaper accounts of various papers from the Society's program were read. "Senator

Sorghum,'' impersonated by H. H. Whetzel, interrupted the dinner and engaged in a conversation with toastmaster Barss. He related at some length humorous experiences he had had with some of the members.

Dr. and Mrs. John Monteith and Dr. Zaumeyer then presented slides and motion pictures. A set of slides showed deceased charter members. One reel revealed charter members in action and a second depicted ''unknown species'' at their various occupations. Dr. L. M. Massey and the committee that assisted him with the dinner program received the thanks of the Society in a resolution passed at the last business meeting.

OFFICERS AND REPRESENTATIVES

The following officers were chosen:

President, R. J. Haskell, United States Bureau of Plant Industry, Washington, D. C.

Vice-President, H. S. Fawcett, Citrus Experiment Station, Riverside, California.

Secretary-Treasurer, F. C. Meier, United States Bureau of Plant Industry, Washington, D. C.

Councilor (two years), M. W. Gardner, Indiana Agricultural Experiment Station, La Fayette, Indiana.

Editor in Chief of Phytopathology (three years), H. B. Humphrey, United States Bureau of Plant Industry, Washington, D. C.

Editors (three years), J. W. Roberts, United States Bureau of Plant Industry, Washington, D. C.; A. J. Riker, University of Wisconsin, Madison, Wisconsin; H. M. Quanjer, Institut voor Phytopathologie, Wageningen, Netherlands.

Associate Editors (three years), D. L. Bailey, University of Toronto, Toronto, Canada; G. H. Godfrey, Association of Hawaiian Pineapple Canners Experiment Station, Honolulu, Hawaii; M. W. Gardner, Indiana Agricultural Experiment Station, La Fayette, Indiana; Miss Nellie Brown, United States Bureau of Plant Industry, Washington, D. C.

Business Manager (one year), F. C. Meier, United States Bureau of Plant Industry, Washington, D. C.

Advertising Manager (one year), J. F. Adams, Agricultural Experiment Station, Newark, Delaware.

Representatives on the Council of the American Association for the Advancement of Science (one year), D. Reddick, Cornell University, Ithaca, New York; C. W. Edgerton, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Member of the Board of Governors of the Crop Protection Institute (three years), I. E. Melhus, Iowa State College of Agriculture, Ames, Iowa, reappointed. The other two members of the Board are: J. F. Adams and H. W. Anderson.

Member of the Permanent Committee on Necrology, A. G. Johnson, United States Bureau of Plant Industry, Washington, D. C., to replace L. R. Jones, resigned.

The following temporary committees were appointed to serve throughout the meetings:

Auditing Committee, F. M. Blodgett, H. A Rodenhiser, and A. J. Mix.

Committee on Elections, E. L. Nixon and W. D. Valleau.

Committee on Publicity, F. C. Meier, W. A. Whitney, and F. L. Drayton.

Resolutions Committee, J. H. Craigie, G. F. Gravatt, and J. C. Gilman.

REPORT OF THE SECRETARY-TREASURER, 1928

The Society started the year 1928 with 750 members. During the past twelve months we have reinstated 2 and lost 33, 20 by suspension for non-payment of dues, 9 by resignation and 4 by death. This makes a net loss of 31 and a total membership at the close of 1928 of 719. Of these, 82 are life members, paid up in full, 30 are life members, pay-

ing currently, and 607 are regular annual members. At this meeting, the secretary has 75 applications for membership and these, if elected, will bring the total membership up to 794, a net gain of 44.

The State of New York again leads this year in the securing of new members with nine applications, Wisconsin comes second with six, followed closely by Iowa and California with five each, Indiana and Minnesota with three each, Florida, Georgia, Louisiana, New Jersey, and Nebraska with two each, and eight other States with one each.

The geographical distribution of our present 720 members in good standing, at the present time, is of interest. Residing within the United States there are 590, in Canada 45, Japan 24, Australia 9, England 6, Holland 5, China, Germany, and Haiti, each 4, France 3, India, Ireland, Norway and Russia each 2, and eighteen other countries, one each.

It is significant to note that Japan has as many members as the Empire of Great Britain including all of its colonial possessions, exclusive of Canada, and that of the new foreign members to be elected at this meeting, eleven are from Japan and three from Great Britain, exclusive of Canada.

Statement of Accounts for 1928, as of December 17, 1928

Receipts:			
Balance from 1927		$1,456.28	
Annual dues: 1926	$ 4.00		
1927	28.00		
1928	1,142.79		
1929	1,699.85		
		2,874.64	
Excess dues		1.12	
Interest on checking account		37.08	
Interest on time deposit		10.00	
Time deposit returned to checking account		500.00	
Sales received with dues		1.75	
Total receipts to be accounted for			$4,880.87
Expenditures: (As per vouchers attached)			
Member subscriptions transferred to PHYTOPATHOLOGY		$1,973.00	
Secretarial work		195.25	
Postage stamps and stamped envelopes		43.74	
Stamped printed envelopes		56.52	
Printing (abstracts, preliminary announcements, ballots, nomination ballots, final program)		123.86	
Expenses of Secretary-Treasurer at Nashville meeting		85.00	
Expenses of representative on National Research Council		36.89	
Check returned by bank		5.00	
Balance as shown by bank statement attached		2,361.61	
Total expenditures and balance			$4,880.87

The available working balance of $674.74 for 1929 should be ample to care for all ordinary expenses. Should the Society wish to make appropriations, they can probably do so to the extent of about $150.00 without interfering too much with the regular work.

For the past six years, the Secretary-Treasurer has been assisted in his work for the Society by Miss Mary G. Van Meter. Over her desk most of the detail has passed and on her shoulders a large share of responsibility has rested. I have been especially fortunate,

Supplementary Statement:

Balance as above stated			$2,361.61
Amount of above receipts due PHYTOPATHOLOGY:			
For member subscriptions, 1929	$1,197.00		
For member subscriptions, 1926, 1927, 1928	45.00		
For sales received with annual dues	6.87		
		$1,248.87	
Amount due sinking fund:			
For 1925, 1927, 1928	306.00		
For 1929	132.00		
		438.00	
			1,686.87
Available working balance for 1929 expenses			$ 674.74

and I think the Society has been fortunate, in having such an efficient and interested helper who has the welfare of the Society so much at heart. I happen to know that she did not submit any bills for her work during two months of this year because she knew that the PHYTOPATHOLOGY account was becoming rather dangerously low. In closing my term as Secretary-Treasurer, I would like to express and to record my high appreciation for the excellent service she is rendering.

Since this may be the last opportunity I shall have at this meeting, I wish to say that I have enjoyed and benefited by my two terms as Secretary-Treasurer of this Society. I appreciate the honor and the opportunity for service that you have given me.

Respectfully submitted,

R. J. HASKELL, *Secretary-Treasurer.*

REPORT OF THE BUSINESS MANAGER OF PHYTOPATHOLOGY FOR 1928

Statement of Accounts for 1928, as of December 17, 1928

Receipts:

Balance from 1927		$ 585.08	
Subscriptions ($498.35 for 1929, $5.00 for 1930)		2,269.30	
Sales, PHYTOPATHOLOGY	$745.57		
Phytopathological Classics	2.59		
		748.16	
Advertising: 1927	237.86		
1928	870.75		
1929	27.16		
		1,135.77	
Interest on Sinking Fund and investment		322.03	
Member subscriptions for 1928		1,973.00	
Donation to Endowment Fund		25.00	
Sinking Fund investment paid up (reinvested)		2,250.00	
Surplus investment released to checking account		1,000.00	
Tropical Plant Research Foundation for colored plate		496.24	
Total receipts to be accounted for			$10,804.58

Expenditures:

Manufacturing PHYTOPATHOLOGY:		
Vol. XVII, No. 11	$ 276.59	
Vol. XVII, No. 12	329.38	
Vol. XVII, Index	127.93	
	$ 733.90	

Expenditures:

Vol. XVIII, No. 1	$1,131.47		
Vol. XVIII, No. 2	501.85		
Vol. XVIII, No. 3	416.83		
Vol. XVIII, No. 4	395.29		
Vol. XVIII, No. 5	620.45		
Vol. XVIII, No. 6	429.17		
Vol. XVIII, No. 7	439.21		
Vol. XVIII, No. 8	560.49		
Vol. XVIII, No. 9	1,048.81		
Vol. XVIII, No. 10	420.56		
Cuts for Vol. XVIII	739.38		
		$6,703.51	
			$7,437.41
Expenses of Editor in Chief			283.48
Postage, Editor in Chief			11.51
Secretarial work, Business Manager			94.00
Insurance on back volumes of PHYTOPATHOLOGY			47.50
Postage in mailing back volumes			11.10
Checks returned by bank			16.00
Sinking Fund reinvested			2,262.04
Duplicate subscription refunded			5.00
Balance as shown by bank statement attached			636.54
Total expenditures and balance			$10,804.58

Last year, at this time, I reported a cash balance of $585.08 plus $1,000.00 of surplus funds invested in securities. This year, as just stated, the cash balance is $636.54 and the $1,000.00 surplus has been spent. We are, therefore, not so well off this year as we were last by $948.54.

Expressing the situation in another way; the actual income for the year 1928 was $6,999.07, while the entire cost of publication for the same period was $7,918.04, or $918.97 in excess of the income. No business can continue indefinitely at that rate and so, in 1929, steps must be taken to bring the expenditures within the budget. It seems evident that we must either shorten up the harness to fit the horse, that is, reduce the size and cost of the Journal to fit the income, or we must get a larger horse. I hope that action will be taken at this meeting which will help to remedy this situation. Even if steps are taken, it is probable that the increased revenue will not begin to come in until next year and so an even closer censorship and limitation of articles submitted seems to be on the program for 1929.

Sinking Fund

The sinking fund, to which six dollars of every sustaining life-membership payment is now added regularly, totals $5,688.00. Of this $5,250.00 is now invested in first-mortgage notes as follows: 1 not of $1,000.00 at 6½%, 2 notes of $1,000.00 at 6%, 4 notes of $500.00 at 6%, and 1 note of $250.00 at 6%. The remaining $438.00, which has but recently been received, is in the Society's checking account and has not as yet been invested.

Endowment Fund

Two members have assumed that there is going to be an endowment fund and have already sent in their checks for twenty and twenty-five dollars, each.

Phytopathological Classics

In December, 1926, 1,000 copies of *Phytopathological Classics*, No. 1, were manufactured at a cost of $232.90. To date, $114.77 has been realized from the sale of slightly more than one-quarter of them, 279 copies to be exact. The remainder (721 copies) are available at forty cents, each.

Status of Subscribers

The present edition of PHYTOPATHOLOGY is 1,575, of which 1,220 copies are being mailed out. The number of subscribers in good standing on December 31, 1928, is 470 (173 domestic and 297 foreign), a gain of 50 for the year. This is a very satisfactory increase—the largest during my experience as business manager and I think probably the largest in the history of the Journal.

Respectfully submitted,
R. J. HASKELL, *Business Manager.*

REPORT OF THE EDITOR IN CHIEF OF PHYTOPATHOLOGY

Volume 18 of PHYTOPATHOLOGY contains 1,030 pages, comprising 75 articles, 26 notes, 3 reports, and 3 book reviews; 29 plates and 142 text figures. From January 1 to December 1, 1928, 114 manuscripts were submitted, of which 19 were returned for revision, and 5 rejected.

Four months has been the average length of time required for publication of papers. Manuscripts submitted during late summer and fall were delayed somewhat on account of the large amount of material on hand. They were edited promptly and were ready for publication in Volume 18, but, because of lack of funds, it was not thought wise to make the numbers larger. As will be seen from the Treasurer's report, the first nine numbers exhausted the funds available for printing PHYTOPATHOLOGY. Otherwise, more material could have been published, and some of the manuscripts would have appeared earlier.

Volume 18 is the largest volume yet published. It is not, however, necessarily any better than some of the thinner volumes. Some of the articles in Volume 18 undoubtedly are mediocre, others are moderately good, and some excellent. Some of the excellent ones were longer than necessary. Conciseness is very desirable, but the problem of attaining it cannot be solved in a short time. The editor of a democratic journal such as PHYTOPATHOLOGY can not reject summarily all manuscripts which are somewhat too prolix; neither has he time to rewrite the articles, himself. In fact, it should not be necessary. But that is what a few contributors seem to expect. Yet the subscribers are paying for prolixity: they are paying more for the Journal than would otherwise be necessary. The problem is not so simple as it might seem to those who have not had editorial experience. It is the natural desire of an editorial board to publish good papers rather than to have them published in other journals; therefore they often are accepted despite the fact that they are unnecessarily long. The idea of many contributors regarding condensation is to eliminate a few adjectives and one or two commas. As has been pointed out repeatedly, writers very often are so impressed with the mental processes by which they arrive at their conclusions that they seem quite incapable of putting themselves in the mental frame of mind of the readers. In other words, they are writing a sort of mental autobiography instead of making a clear, concise presentation of their results for the benefit of their readers. The difficulty is a fundamental one. It seems quite likely that the best method of solution is to impress upon all contributors the necessity for having some critical colleague read and comment on their manuscripts

before they are submitted for publication. This would be far better than putting the burden of revision on the editor. It is very seldom that the editor has an opportunity to discuss personally with an author the shortcomings of a paper. It is difficult to adjust many things regarding manuscripts by correspondence, whereas it is relatively easy to do so in personal conference. Furthermore, most editors sympathize with an author's desire to retain his own style. Therefore the author himself should make his own revision. Many organizations now follow the plan of having some one within the organization criticize manuscripts before they are offered for publication. The results are gratifying, indeed, to an editor, and probably also to the readers of the papers.

When the number of plates in Volumes 16, 17, and 18 is compared with the respective number of pages in these volumes, a reduction in the number of plates will be noted. A still further reduction in the number should be possible, as the paper on which PHYTOPATHOLOGY is printed is suitable for text figures. In addition to saving space, text figures have the added advantage of convenience of position. When figures are not too large to be presented on one page with the legend, they should be prepared as text figures. This applies also to combinations of small figures or drawings.

A reduction in the total number of illustrations would be highly desirable. After all, there is no particular object in publishing a picture which does not add something to the article. Most contributors have been very helpful in cooperating with the editors in the selection of the pictures to illustrate their papers; others, however, have attempted to insist that their articles be veritable picture books. It might be well to remind all contributors that it costs money to reproduce photographs and drawings. As a matter of fact, it also costs money to print words. Plates and tabular material are especially expensive. Possibly the average contributor is too little concerned with the financial problems of publication to realize that conciseness is desirable not only from the standpoint of clear presentation but that it is quite necessary also from the standpoint of economy.

There is urgent need for additional funds for publication. The Endowment Committee is submitting a report at the present meeting. If this report is adopted, and the provisions put into effect, the future of PHYTOPATHOLOGY should be reasonably well assured. With the additional funds it should be possible to publish more good papers, and the editor will not be under the necessity of insisting on too great conciseness, which sometimes is incompatible with a clear exposition of the facts presented. It seems particularly desirable that provision be made for publishing a number of good colored plates to illustrate some of the better articles and also to publish viewpoint papers and papers summarizing the present status of knowledge. In the past it has been difficult if not actually impossible to accept such papers. With adequate financial support, PHYTOPATHOLOGY should become a better and more useful journal.

E. C. STAKMAN, *Editor*.

REPORT OF THE ADVERTISING MANAGER

The advertisements for 1928 show a decrease of six pages compared to the total number for 1927. In the twelve issues of PHYTOPATHOLOGY for 1928, there was a total of 102 advertisements divided as follows: 32 one-page, 53 one-half page, 16 one-fourth page and 1 one-eighth page advertisements, or a total equivalent to 62⅝ full-page advertisements.

It is further urged upon members to consider that advertising returns are an important factor in the maintenance of PHYTOPATHOLOGY. Members can cooperate by mentioning to representatives of various companies with whom they come in contact the practical medium PHYTOPATHOLOGY offers through its advertising space. The total

purchases of supplies, equipment, etc., by our membership represents a large sum of money distributed to various commercial houses. It seems reasonable to expect in return a sympathetic as well as a financial support through advertising. However, this opinion will count for little unless each member assumes the responsibility of imparting the message. If members would interest themselves and assume a responsibility along this line, it would materially help to increase the advertising revenue and thus assist in meeting the total expense of publishing PHYTOPATHOLOGY.

J. F. ADAMS, *Advertising Manager.*

REPORT OF THE ADVISORY BOARD

The personnel of the Board for 1928 consisted of F. D. Fromme, Chairman, representative at large; J. G. Dickson, Secretary, representing the Midwest; E. L. Nixon, representing the Northeast; B. B. Higgins, representing the South; S. M. Zeller, representing the West; J. A. Stevenson, representing the United States Department of Agriculture; F. L. Drayton, representing Canada; and I. E. Melhus, representative at large.

Summer Meetings. The summer meeting of 1928 was devoted especially to the diseases of ornamental plants and was under the immediate direction of a committee composed of W. H. Martin, Chairman, W. H. Rankin, L. O. Kunkel, C. R. Orton, and L. M. Massey. The dates were August 20 to 22, inclusive, and the itinerary included visits to nurseries in New Jersey, a study of the experimental work in progress at the Boyce Thompson Institute at Yonkers, New York, and at the New York Botanical Garden, and an inspection of the work of the Bartlett Research Laboratory at Stamford, Connecticut. A number of estates in the vicinity of New York City were visited by the group. The meeting was well attended and was a most successful one from all standpoints.

National Research Council. The Board continues to function through its representative in an advisory capacity with the Division of Biology and Agriculture of the National Research Council. The accomplishments and activities of the Division for 1928 which are of particular interest to the membership of the Society are summarized briefly in the following statement. The Committee on Research Publications has been reorganized under the chairmanship of B. M. Duggar and has held several informal meetings during the summer. It is cooperating with the Committee of the National Academy of Sciences with the view of determining and satisfying the needs of scientific biological journals in the publication of original research.

In addition to the fellowships in the Biological Sciences which the Council has operated since 1923, it is proposed that fellowships in forestry and agriculture be established and a plan for instituting such fellowships has been presented to an interested Foundation for its consideration. The Committee on sulphur fellowships was discharged during the year inasmuch as its work is now completed. The contributing sulphur company has been convinced of the limitations to the utilization of sulphur for direct application to the soil because of the negative character of many of the results. The seed-germination fellowship also has been completed.

The question of the establishment of a Graduate School of Tropical Agriculture has been rather definitely determined by the Porto Rican Government and Cornell University under a plan of cooperation similar to that of the Graduate School of Tropical Medicine.

The Book on the North American rusts has gone to press.

Continued progress at the Barro Colorado Island Biological Laboratory is reported. Additional buildings, new trails and other improvements have been made and the number

of scientific workers more than equals the expectations of those who planned this station.

The Commission of Standardization of Biological Stains is preparing a book on the uses of biological stains to be published by the Chemical Foundation. The Chairman of the Commission reports that six new dyes have been certified during the past year.

The Division has established three new committees to correlate work in three very different lines of activity. They are as follows: National Finance Committee on Plant Nomenclature; Committee on the Provision and Preservation of an Adequate Supply of Experimental Animals and Plants for Investigations; and the Committee on Investigation of the Effects of Radiation upon Organisms. The activities of these committees will no doubt be of interest to plant pathologists as well as to workers in other fields of Biological Sciences.

F. D. FROMME, *Chairman.*

REPORT OF THE COMMITTEE ON ENDOWMENT FOR PHYTOPATHOLOGY

The Committee recognizes the immediate need of additional funds for publishing PHYTOPATHOLOGY, and also is of the opinion that additional money should be available for safeguarding the future. That additional money is required immediately to maintain the standard of PHYTOPATHOLOGY is quite evident. It has been necessary for the editorial board to insist on greater conciseness than sometimes is desirable in the manuscripts submitted for publication. It has been necessary, also, to be quite economical with respect to the number of illustrations. Not only that, but it has been necessary to reject some valuable viewpoint papers and summaries which would constitute valuable contributions to phytopathological literature, because it seemed that the results of research should receive first consideration. In spite of all precautions which were taken to publish only the results of research, the funds set aside for the year were exhausted in publishing nine numbers, leaving three to be paid for from next year's budget. That means that we either will have to reduce the size of the volume of PHYTOPATHOLOGY by about 25 per cent or will have to secure additional funds. It seems desirable to secure additional funds. The Committee therefore makes the following recommendations:

Immediate needs

1. That the annual dues and subscription for PHYTOPATHOLOGY be increased one dollar, the additional money to be allotted for publishing PHYTOPATHOLOGY;
2. That the drive for life memberships be continued.

Future needs

3. That an endowment fund be established, to be known as the George Richard Lyman Memorial Fund for Phytopathological Publications.

In explanation of these recommendations the Committee wishes to submit the following:

The annual dues and subscription for PHYTOPATHOLOGY should be increased in order more nearly to meet the cost of publication. The price now is ridiculously low, and it seems only fair to increase it. The additional income thus obtained should be made available immediately, to meet present needs in publication.

It seems good business to continue the drive for life memberships.

While the increased subscription and the income from life memberships will help finance the publication of PHYTOPATHOLOGY at present, the Committee is strongly of the opinion that there should be some insurance for the future. It strongly recommends, therefore, that an endowment fund be established, the interest of which will be used for PHYTOPATHOLOGY or other phytopathological publications. It seems peculiarly fitting that this fund be named the George Richard Lyman Memorial Fund, in accordance with

the suggestion made by Dr. Haskell. During his lifetime, Dr. Lyman gave so unselfishly of his time and energies to further the interests of the American Phytopathological Society and PHYTOPATHOLOGY that it seems very appropriate to establish the endowment fund as a tribute to his memory. The Committee recommends that the endowment fund be raised by voluntary subscriptions from the membership of PHYTOPATHOLOGY and by voluntary gifts from people of means who may be induced to contribute. In order to get the fund started it is recommended that the membership of the Society be circularized immediately for the purpose of inviting contributions. It is recommended, further, that hereafter the secretary-treasurer invite contributions to the fund at the time the statement of annual dues is sent out.

The Committee recommends the adoption of the report.

J. G. BROWN,
L. R. HESLER,
A. J. RIKER,
H. H. WHETZEL,
E. C. STAKMAN, *Chairman.*

COMMITTEE ON EDITING PHYTOPATHOLOGICAL ABSTRACTS

The manuscripts submitted this year show a marked improvement over those of previous years in two respects: They were for the most part within the 200-word limit, and they were sent in promptly. The final action of the Committee was to accept all abstracts which were in the mail on November 1, and, in two instances where papers were sent from outside this country, to accept an advance notice and the title of the paper in lieu of the complete draft, provided the complete paper was in hand by November 20. Only a few papers were rejected on account of tardy submittal. For most of these, there was no apparent reason for lateness other than that preparation was not begun in time, but two or three authors claimed extenuating circumstances. The Committee thought it best to make no exceptions in fairness to all who submitted their papers on time, though this was a difficult decision and was taken contrary to personal desires and despite the interest and value of certain contributions.

The Committee feels that the question of reverting to November 15 as the final date for acceptance of abstracts should perhaps be reconsidered. The regulation adopted last year was necessary for two reasons: (1) evidence of a growing tendency to submit manuscripts without much regard for the announced closing date, and (2) manuscripts frequently exceeded the prescribed length. The Committee obviously could not undertake, in condensing 400 words to 200, satisfactorily to present the author's viewpoint, and there was not enough time to refer such papers to the authors for revision.

Please bear in mind that in order to provide sufficient time for printing and distributing the abstracts in advance of the meetings, the manuscripts must be completely edited and the program arranged by about November 20. Most of the manuscripts submitted require only minor editorial attention, but there are some such that the Committee could do no greater affront to the authors than to publish them in their original form.

We propose for the ensuing year and thereafter, until further notice, that November 15 be designated as the closing date for the receipt of manuscripts for phytopathological abstracts and that this be interpreted strictly to mean that the abstracts must be in the hands of the Committee on or before that date.

Late receipts of manuscripts and length in excess of 200 words will act automatically to disqualify. In determining the number of words, the following procedure will govern: (1) The title will not be counted, nor will the article "a" in the text of the ab-

stract; (2) numerals of whatever magnitude will count as one word. In expressions of temperature, the abbreviation °C. (F.) will count as one word together with the numeral. (3) Latin binomials, including the authority, will count as one word, also common names of hosts when compound; as durum wheat, sweet potato, Japanese honeysuckle. However, authorities should not be cited in connection with scientific names unless there is real ambiguity in the use of a binomial without the authority.

Manuscripts presenting material which, in substance, has already been published will not be accepted.

The Committee also offers the following suggestions to authors to aid in the preparation of suitable abstracts.

1. Make the titles brief. Omit such introductory statements as "Studies on," "investigations of," "progress report on."
2. Write the title in lower-case letters except for the initial letter of the title and of proper names. Underscore with a solid line to indicate italics. Write the author's name in solid capitals underneath the title. In the third line, indicate the time desired for presentation and whether lantern projection is to be used. Double space the text and avoid excessive paragraphing.
3. Tables should not be used in abstracts;—they require special setting and often waste space. Their place is in finished papers. Tables may rightly be used in connection with the presentation of papers by projecting them from lantern slides.

In all enumerations of weight, measure, percentage, degrees and proportions, use numerals instead of spelling out. In other cases, spell out amounts of ten or less, and use numerals for larger amounts, but give consistent treatment to amounts in connected groups.

Write out grams, pounds, bushels, inches, per cent, but cm., mm., kg., p.p.m. (parts per million) may be employed.

The purpose of an abstract is to present clearly and succinctly the principal results and conclusions from a piece of work. The reporting of many results in detail is likely to be confusing; and since, ordinarily, an abstract is presumed to be the forerunner of a paper, it is unnecessary to present the same details in two places. On the other hand, speculation and inadequately supported generalizations have no place in an abstract. Excellent models of abstracts, both as to selection of material and manner of preparation may be found in the *Review of Applied Mycology*, and many good examples may be found also in *Biological Abstracts*.

Finally, the Committee believes that all members of this Society should assume a moral obligation to present their papers in person or withhold the abstracts.

FREEMAN WEISS (*Chairman*),
JOHN W. ROBERTS,
HARRY B. HUMPHREY,
ANNIE R. GRAVATT.

COMMITTEE ON INVESTIGATION OF FOREIGN PESTS AND PLANT DISEASES

With the development of country-wide agencies for determining the losses suffered in agriculture from insects and diseases has come a consciousness of the tremendous tax that this industry and, with it, the rest of the Nation must bear to make up for the damage done by our multitude of crop parasites. It has been conservatively estimated that our plant products are depleted to the amount of 25% annually by the inroads of insects and diseases, and it is most significant that by far the greater part of this tax on our

national economy must be attributed to pests of foreign origin. A decade or more ago the hope was cherished that, by means of a quarantine system sufficiently broad in scope and effectively administered, the host of invaders still waiting to reach our continent might be kept out with a completeness that would enable us to develop our agriculture on a permanent and stable basis. But, along with the rapid advancement in transportation methods, and large and constant increase in world-wide travel and traffic, together with the demand for foreign goods brought about by a higher general standard of living, the rising flood of materials from abroad has swelled to a point where the quarantine dam, however well constructed, seems insufficient to protect the country indefinitely from further invasion. Your committee feels that it is voicing a widespread conviction in expressing the opinion that permanent exclusion of foreign pests can scarcely be regarded as attainable and that sooner or later foreign insects and fungi are likely to be spread as far as food, climate, and natural enemies will permit.

This belief by no means implies that an open-door policy should be established. It is decidedly to our advantage to delay all unwelcome immigrants as long as possible, not only because each imported parasite lessens in a permanent way the productive capacity of the Nation, but also because each intruder disturbs the balance of agriculture, necessitating constant and costly readjustments in this industry which is perhaps the least adaptable of all to sudden change.

But, while all quarantine effort should be maintained or even strengthened, it may be expected that from time to time some of the dangerous parasites from other parts of the world will slip past the guard line and establish themselves in our midst. With the danger of such leakage constantly before us, it would seem obvious that the foresighted and profitable thing to do would be to study these prospective pests in their place of origin; an intimate knowledge of habits, life histories, means of dissemination, and methods of control might enable us not only to guard against, or at least delay, their introduction here, but if they should finally enter this country, there will be at once available a body of knowledge to enable us to cope with them immediately instead of devoting precious years to devising ways and means of control. Surely in a matter involving the basic food supply of our rapidly increasing population we may rely on the far-visioned interest of our national leadership to take these simple precautions for safeguarding its future.

Your committee notes that the necessity for investigation of this kind has already been recognized by the Federal authorities at Washington and that, on several occasions, studies of foreign parasites, already introduced or not yet introduced into the United States, have been undertaken by them. It is urged that steps be taken to formulate and put into effect a definite, permanent, and adequate program for the purpose of making such studies.

Your committee further suggests that, while colleges and private research institutions should be encouraged as far as possible in making contributions to our knowledge of foreign pests and their control, yet it would appear that leadership in this field should rest with the Federal Government since it has above all other agencies a wide experience, a deep understanding of the subject from many angles, and can most readily command both staff and financial support on a scale large enough to make the program effective. Moreover, in matters of this nature, arrangements with foreign governments are concerned and the Federal authorities are in a better position to deal with such relations than private institutions.

Your committee further recommends that this Society bring these considerations to the attention of the proper Federal authorities with an earnest request, first, that ade-

quate provisions be made for their investigation in their native habitat, in as wide a manner as possible, of insects and diseases which, because of geographical, crop, or commercial conditions, are likely to be introduced here; second, that such investigations shall include the collection of all information relating to these insects and diseases which might enable us to prevent or delay their entry here or in case of their accidental introduction at least to provide effective and immediate control; third, that in conformity with this policy, steps be taken to carry out such investigations on the following:

1. Diseases of corn, with special reference to mosaic of Hawaii which is stated by a foremost authority to be the most serious disease of this crop yet known.
2. Investigations of Fiji disease of sugar cane in the Philippines and South Sea areas.
3. Diseases of wheat, with special reference to stripe rust, known to be serious in Europe.
4. Oriental citrus diseases centered around the black-spot disease, *Phoma citricarpa*.
5. Larch canker.
6. Elm disease of Europe.
7. Soy-bean diseases in the Orient and Manchuria.
8. Certain apple diseases known to be serious in N. Japan:
   Diaporthe mali.
   Sclerotium mali.
   Valsa mali.
9. *Botrytis liliorum* in Japan.

Appendix I.

Your committee hesitates to present any detailed plan for the further organization of this program, having in mind that these matters are closely bound up with budgetary and administrative limitations and procedure, but it desires to present the following suggestions as to features in this program which ought to be given due consideration:

1. The type of investigations herein urged should be carried out primarily on a rather definite crop or project basis rather than as an attempt to collect general information over the whole range of pests in other parts of the world.
2. At the outset, attention should be centered on those localities which, because of trade volume, presence of known pests, or other considerations, may be considered as danger points.
3. Such investigations should cover host range, nature and extent of damage, life-history data, biological and artificial control, methods, means, and materials by which the parasite might be brought to this country, and such other features as might have a bearing on exclusion in the first place or control in case of accidental introduction.

Appendix II.

List of important introduced pests. (Not printed.)

Appendix III.

List of cases in which studies of diseases have been made by this country in foreign lands. (Not printed.)

C. R. ORTON, W. A. MCCUBBIN, F. D. FROMME.

REPORTS OF OTHER COMMITTEES AND REPRESENTATIVES

Phytopathological Classics. In 1927, the editors got under way plans for the preparation of two additional classics. One, the bringing together of the papers of Sir Joseph Banks on his early rust work in England by Mr. J. W. Sinden, of Cornell, and two, a translation of Tillet's early work on the smuts by Dr. H. B. Humphrey.

These manuscripts were not urged for publication at all during 1928 due to the statement by the treasurer that, in his opinion, the finances of the Society did not warrant their publication at the present time. However, both of these manuscripts can be prepared for the printer in a relatively short time when funds are available for that purpose.

H. H. WHETZEL, *Chairman.*

Representative on the Council of the A. A. A. S. Dr. Reddick reported:

1. That members of this Society are eligible to join the American Association without paying the customary entrance fee $5.00. This practice will be continued for another year but it is not yet an established policy and eventually may be abandoned.

2. That members of the American Association who are professionally engaged in scientific work are eligible for fellowship, and any such member who is not a fellow would be recommended for fellowship if the member would send him a memorandum stating merely that he is not a fellow.

3. That the Association has imposed a registration fee to help pay the extraordinary expenses of the meetings rather than to allow this expense to fall upon the "local committee," as has been the case in the past. It is unlikely that the money received in this way will pay all of the extraordinary expenses.

4. That the Association has adopted the *certificate* plan of railway ticket validation in preference to the *identification* plan as the one which is the simpler and more universally adapted to existing conditions. Some railroad companies positively require some means of identifying purchasers of special-rate tickets, others may not.

Representative on American Type Culture Collection. C. L. Shear submitted the following report:

A summary of the year's work shows a healthy growth in the activities of the collection. The collection contains more cultures and is in better shape than at any previous time. The output of cultures has increased during the year, the total being 4,761, as compared with 3,897 last year. Of the 4,761 cultures, 4,281 were bacteria, 260 fungi, and 220 yeasts. The largest part of the cultures have been furnished for teaching purposes, but a good many also for use in investigative study. Of the total, 4,178 have gone to institutions, 156 to individuals, and 427 to commercial dealers in biological supplies.

A second edition of the *Catalogue of Cultures* is ready for distribution. It includes about 650 cultures acquired since the first edition was printed and from it have been omitted about 150 cultures which had been lost or had been discontinued because impure or not true to type.

Auditing Committee. This is to certify that we have examined the books of the Secretary-Treasurer of the American Phytopathological Society for 1928 and find them to be in excellent condition. We found the subvoucher and deposit slips accounting for all the expenses and receipts, as well as canceled checks and monthly bank statements, to be all in excellent order and believe the same to be correct.

F. M. BLODGETT and H. A. RODENHISER.

Resolutions Committee. A committee consisting of J. H. Craigie, G. F. Gravatt and Joseph C. Gilman brought in the following resolutions all of which were adopted except the last, number 8.

> *Resolved:* That the Society thank the members of the General Local Committee of the American Association for the excellent arrangements provided for this meeting. Particular mention should be made of the services of Dean G. B. Pegram and Dr. S. F. Trelease in the matter of places of meeting and to Prof. M. A. Bigelow for the meeting, room arrangements.

Resolved: That the Society thank the retiring Secretary-Treasurer, Dr. R. J. Haskell, and the Editor in Chief, Dr. E. C. Stakman, for the unstinted generosity of time and energy which both men have given during their terms of office.

Resolved: That the Society express its thanks to the members of the Society in charge of the entertainment at the annual dinner, especially to Dr. L. M. Massey and to Mr. and Mrs. Monteith, and Mr. Zaumeyer for the films.

Resolved: That the Society express and record its high appreciation for the excellent services rendered by Miss Mary G. Van Meter in the office of the Secretary-Treasurer and Business Manager.

Resolved: That the Society appoint a committee to inquire into the desirability of seeking to develop an international organization for the furtherance of our science and the promotion of international cooperation and good will.

Resolved: That the members of the American Phytopathological Society recognize the great service that is being rendered by *Biological Abstracts* and would recommend that the President appoint a committee to cooperate with the Board of Editors of this Journal and to report to the Society at its annual meeting.

Resolved: That the Society authorize the appointment of a standing committee on plant, quarantine and regulatory work to keep the members in touch with progress made each year along these lines. One of the functions of this committee would be to consider the advisability of regulating the importation of pathogenic cultures.

The following proposed resolution failed to pass:

Resolved: That the Society authorize the appointment of a committee to investigate the possibilities of securing the establishment of a division of fundamental research in fungicides (and perhaps insecticides) by the Federal Government.

Permanent Committee on Necrology. The decease of five members is reported: Owen F. Burger, Frank G. O'Donnell, Flora W. Patterson, Ralph C. Thomas, and Tōji Nishida.

On account of their achievement and personal qualities, these members had won the professional respect and personal friendship of their colleagues. The American Phytopathological Society records its sense of loss in the death of these members. The formal report will be found at the end of this record.

L. R. JONES,
G. P. CLINTON,
M. B. WAITE.

ACTION OF THE COUNCIL

In addition to making the appointments mentioned in the first part of this report, the Council made the following recommendations which were approved by the Society.

1. That the incoming and outgoing officers prepare a revision of the Constitution, by-laws, and list of members to be presented at the annual meeting of the Society.

2. That Article 5 of the Constitution be amended by changing the word "beginning" in the next to the last sentence to "end." The sentence will then read, "The term of service of a Council member from a Division shall commence at the end of the annual meeting of the Society next following his election by the Division."

3. The council presents for the consideration of the Society the amendment of standing rule number 6 to read as follows: "Unless otherwise ordered, the Secretary and the

President, or, in case of his inability to attend, the Vice-President, are authorized to attend the annual meetings of the Society at the Society's expense.''

4. The Council recommends the acceptance of the resignation of E. C. Stakman, M. N. Levine, and L. R. Hesler as Editors of PHYTOPATHOLOGY.

5. Dr. H. B. Humphrey is extended the opportunity of the editorship of PHYTOPATHOLOGY if he wishes that in preference to the secretary-treasurership to which he has been duly elected. If he accepts the editorship and resigns the secretaryship, it is recommended that F. C. Meier, who received the next highest number of votes for secretary-treasurer be declared elected.

6. It is recommended that thanks be extended to the Advertising Manager for his past services, and that hereafter 10% of the advertising receipts be given the Advertising Manager. It is believed that this will result in increasing the net revenue to PHYTOPATHOLOGY from advertising.

7. The report of the Endowment Fund Committee is approved and the adoption of the methods proposed for increasing the income of PHYTOPATHOLOGY is recommended to the Society.

8. It is recommended that the Committee on revision of the Constitution investigate the question of the appointment of trustees to care for the sinking fund and the endowment fund of the Society.

9. It is recommended that subscription rates for PHYTOPATHOLOGY be increased one dollar per year and that the reduced rates to European subscribers be discontinued.

10. The incoming President is authorized to appoint as delegates any members who may be attending the Fourth Pacific Science Congress in Java, May, 1929.

OTHER BUSINESS

The reports of officers, committees, and representatives, as printed above, were accepted. The report of the Committee on Foreign Plant Diseases and Pests and that of the Committee on Editing Abstracts were ordered printed in the proceedings of the meeting.

Dr. Donald Reddick reported that those desiring information concerning transportation and general plans for the 1930 International Botanical Congress in Cambridge, England, can get in touch with Dr. G. H. Coons, Bureau of Plant Industry, Washington, D. C., who is on a general committee of American botanists that has these matters in charge. It was further stated that the arrangements for the sessions on phytopathology at the Congress were adequate and that the fears that they would not be were caused by a typographical error in the early announcement.

It was moved and carried at the second business meeting that the Advisory Board of the Society be abolished and that its functions be taken over by the Council.

Dr. I. E. Melhus extended an invitation to the Society to visit Ames and, if desired, to hold some of our sessions there. Professor Whetzel moved that the invitation be accepted with thanks, and that the matter of holding sessions at Ames be considered by those in charge of the meeting.

The Committees on Endowment Fund and on Foreign Plant Diseases and Pests were ordered continued.

Dr. L. R. Jones moved that the thanks of the Society be expressed to Miss Van Meter and that the Secretary determine the amount of the Society's financial indebtedness to her.

Dr. A. G. Johnson moved a vote of thanks to the retiring Secretary-Treasurer for his services.

R. J. HASKELL, *Secretary.*

IN MEMORIAM

TŌJI NISHIDA

APRIL 15, 1874—JUNE 19, 1927

Tōji Nishida received the degree of Bachelor of Agriculture from the Sapporo Agricultural College (now Hokkaido Imperial University) in 1899 and the degree of Doctor of Agriculture from the same institution in 1919, specializing throughout in mycology and plant pathology.

From 1899 to 1904, he was on the staff of the Imperial Agricultural Experiment Station at Nishigahara. From 1904 to 1914, and again from 1923 to 1924, he served at the Kyushu branch of the Imperial Experiment Station at Kumamoto, except during the period of the Russo-Japanese war when he served in the Japanese Army. From 1914 to 1923, he was plant pathologist and director of the Kobe Substation of the Imperial Quarantine Service. From 1924 to the time of his death, he attended the Kumamoto Medical College with a view of specializing on comparative plant and animal pathology.

FRANK GETCHELL O'DONNELL

OCTOBER 20, 1896—JANUARY 11, 1928

Frank Getchell O'Donnell received the degree of Bachelor of Engineering from Schuylkill Seminary in 1917 and the degree of Bachelor of Science from Pennsylvania State College in February, 1920, specializing in plant pathology in the latter institution.

Previous to and immediately following his graduation from Pennsylvania State College, he was employed by the Pennsylvania Agricultural Experiment Station as Field Assistant in potato-disease investigations. From April 20, 1920, to the time of his death, he was on the staff of the Federal Horticultural Board, U. S. Department of Agriculture, advancing from field assistant to assistant pathologist. With the Federal Horticultural Board his earlier work was on the control of potato wart and his later work was on seed disinfection.

To all who knew him, his life was one of promise because of his broad foundation in science, his ingenuity in adapting or devising methods and apparatus to suit his needs, his uncanny knack of handling pure cultures, his unlimited patience, and his painstaking accuracy in conducting experiments.

OWEN FRANCIS BURGER

JUNE 8, 1885—JANUARY 26, 1928

Owen Francis Burger received the degree of A.B. from Indiana University in 1909, the degree of M.S. from the University of Florida in 1911, another M.S. from Harvard University in 1915, and the degree of Sc.D. from Harvard University in 1916.

From 1909 to 1911 he assisted in plant pathology, Florida Agricultural Experiment Station; from 1911 to 1913 he served in the same institution as assistant plant pathologist; from 1913 to 1916 he held scholarships at Harvard University; from 1916 to 1918 he was instructor in plant pathology, Citrus Experiment Station, University of California; from 1918 to 1920 he was pathologist in the Office of Fruit Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and from 1920 to the time of his untimely death he was head of the Department of Plant Pathology of the Florida Agricultural Experiment Station.

He was the author of many papers dealing chiefly with fruit diseases. He was an able investigator, capable administrator, and excellent cooperator, combining in all a most congenial, jovial, and friendly personality. Truly it can be said that here was one who served with courtesy and consideration for all.

MRS. FLORA WAMBAUGH PATTERSON

SEPTEMBER 15, 1847—FEBRUARY 5, 1928

Flora W. Patterson prepared for her life work as a mycologist at Radcliffe College and the Gray Herbarium. Practically all of her work in her chosen profession was carried on in the U. S. Department of Agriculture during the years from 1895 to 1923, the latter year marking her retirement from active duty. She was, therefore, one of the original and long-time members of the Bureau of Plant Industry.

Mrs. Patterson's work dealt chiefly with the determination and care of the specimens that found their way into her department's herbarium. Such work, while very valuable to others, is time consuming and often prevents the one engaged in it from pursuing outside scientific investigations. Such publications, consequently, that Mrs. Patterson issued consisted of lists of fungi and short articles on fungi of economic or special interest. Her work was largely in the nature of service to others rather than search for individual renown.

Service seems to have been the keynote of her life, as we read the account of it as published by a co-worker. The effective but quiet aid given her family, as well as scientific friends, in the end, counted more than a brilliant personal career. For after all, when the end comes, we measure people by service rendered rather than by their ambitions.

RALPH CLEON THOMAS

APRIL 9, 1892—DECEMBER 25, 1928

Ralph Cleon Thomas received the degree of Bachelor of Science from the Virginia Polytechnic Institute in 1917 and the degree of Master of Science from the same institution in 1919, specializing in agronomy, plant pathology, and botany. From February, 1923, to August, 1923, he took special work in bacteriology at the Michigan Agricultural College. During the summer of 1924, he took special work in plant pathology at Cornell University.

During the first part of the school year, 1917–18, he was student assistant in bacteriology and plant pathology at the Virginia Polytechnic Institute. From March, 1918, to April, 1919, inclusive, he served in the Medical Corps, U. S. Army, having overseas service from July, 1918, to April, 1919. From August, 1919, to November, 1919, he was assistant county agent in Virginia. From November, 1919, to September, 1922, he was extension plant pathologist at the Virginia Polytechnic Institute. From February, 1923, to August, 1923, he was graduate assistant in bacteriology at Michigan Agricultural College. During the school year 1923–24 he was a teacher in Jackson Junior High School, Roanoke, Virginia. From October, 1924, to the time of death, December 25, 1928, he served as agent in the Office of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, working on the diseases of tobacco in the Southern states.

The genuineness of his friendship was indicative of his noble character. Among God's noblemen, none ever was more merciful to his opponents; none more charitable to the needy; and none more thoughtful of the unfortunate and neglected. He was a veritable incarnation of Christianity, and though taken in the flower of young manhood, Ralph C. Thomas will always be alive in the memory of his friends.

MINUTES OF THE FIELD MEETING OF THE SOUTHERN SECTION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, JULY 11–13, 1928

The Southern Section of the American Phytopathological Society assembled at Experiment, Georgia, July 11, 1928, for the summer field meeting. Thirty pathologists from seven States and the District of Columbia took part in the tour, eighteen making the entire trip. After a brief discussion of the plan and purpose of the meeting, the remainder of the morning was spent in viewing and discussing pathological work in progress at the Georgia Experiment Station. At noon, the visitors were guests of Chairman B. B. Higgins at a luncheon served on the station grounds. The afternoon was spent in visiting peach orchards, bean and pepper fields, a cannery near Griffin, Georgia, and some peach-fertilizer plats where the effect of fertilizers on winter injury and on peach bacteriosis was observed. At 6:30 P. M., the visitors were tendered a barbecue by the experiment station.

On the morning of the 12th, the group drove to Fort Valley where Dr. Lee Hutchins demonstrated his work on "Phony" peach and Mr. J. Dunegan discussed peach bacteriosis. After luncheon, the party drove to Sylvester to see a large cantaloupe farm and packing house, and then on to Tifton for the night.

At the business meeting held that evening, it was agreed that another field meeting should be scheduled for 1929 in a territory to be decided upon during the winter. The following officers for 1929 were elected at this meeting for the Southern Section of the American Phytopathological Society:

R. F. Poole, North Carolina, Chairman.

H. H. Wedgworth, Mississippi, Secretary.

B. B. Higgins, Georgia, Representative on Council, Am. Phy. Soc.

Early in the day of July 13, a visit was made to the Coastal Plain Experiment Station where research on cantaloupe and tobacco disease was in progress. Other diseases of special interest were found here. At Thomasville, the visitors were entertained at a delightful luncheon by the local Chamber of Commerce, following which a trip was made to some of the local pecan groves to observe the work on pecan diseases conducted by J. B. Demaree, pathologist in charge of the Pecan Disease Laboratory of the U. S. Department of Agriculture. Led by O. C. Boyd, pathologist, State Board of Entomology, stops were made also at near-by melon fields.

The meeting ended officially here, but a number of the visitors continued to the Tobacco Experiment Station at Quincy, Florida.

The Section tendered a vote of thanks to L. M. Hutchins, John Dunegan, Frank Van Haltern, R. C. Thomas, J. B. Demaree, and O. C. Boyd for the hearty spirit of cooperation shown in planning and conducting the meeting and in arranging to entertain the visitors in their respective localities.

B. B. HIGGINS, *Chairman.*
V. H. YOUNG, *Secretary.*

PHYTOPATHOLOGY

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PHYSIOLOGICAL STRAINS OF *ALTERNARIA SOLANI*[1]

REINER BONDE

INTRODUCTION

It has been shown in previous publications that *Alternaria solani* (E. & M.) J. & G. can produce a tuber rot of potatoes (*Solanum tuberosum* L.) (4, p. 78; 5; 6; 7, p. 6–10; 8; 9) and that the fungus upon its isolation from potato-tuber lesions displays certain differences indicative of the occurrence of more than one strain (1; 5, p. 282; 6). The purpose of this publication is to describe these differences more fully and to discuss some strains which originated (*in vitro*) through saltation in culture. The strains are compared as to spore dimensions, pathogenicity on leaves and tubers, and, chiefly, pigmentation of the potato-agar substratum. Such pigmentation by the fungus in question is referred to by Rands (15, p. 20) but he did not study it particularly.

SOURCES AND VARIABILITY OF THE STRAINS USED[2]

The sources and variability of certain strains, chosen for more careful comparisons, are indicated as follows:

Strain C-1 was isolated in 1924 from a lesion on a Spaulding Rose tuber grown in Maine and shipped to Florida. It has remained stable.

Strain C-2 was obtained as for C-1 except that the shipper and stock were different. It produced a saltation (C-5) distinguished by a darker surface color.

Strain C-3 was isolated in 1924 from an Early Ohio tuber grown in Minnesota and sent to Maine for diagnosis. Saltations distinguished by darker surface color have originated from this strain.

[1] Most of this article is included in a thesis accepted in partial fulfillment of the requirements for the degree of Master of Science at the University of Maine. The writer wishes to make grateful acknowledgment to Dr. W. J. Morse, Dr. W. H. Eyster, and Dr. Donald Folsom, for advice given during the course of this study, and to Dr. Folsom for criticism of the manuscript. It is realized that the scope of these data is limited, but it has not been feasible to supplement them by further studies so that it seems desirable to report the available results without further delay.

[2] The letters C, I, and N, here designate respectively chromogenic, intermediate or variable, and nonchromogenic strains.

Strain C-4 was isolated in 1922 from Spaulding Rose stock grown in central Maine. It has remained stable.

Strain C-5 arose as a saltation (1925) in a culture of strain C-2 and has remained stable.

Strain I-1 was isolated from the same stock as C-4 but from a different lesion. It varies in culture from slightly chromogenic to nonchromogenic, generally coloring the medium pink instead of a deep red or carmine.

Strain I-2 originated as C-4 and I-1 but from a different lesion. It is variable on potato agar and may range from nonchromogenic to chromogenic, producing a distinct carmine.

Strain I-3 originated in 1925 as a saltation in a culture of N-2 and varies in chromogenicity.

Strain N-1 was isolated in 1924 from the same stock as C-2 but from a different tuber. It produces saltations with a more rapid growth rate, which are sometimes slightly chromogenic.

Strain N-2 was isolated in 1922 from the same stock as C-4, I-1, and I-2 but from a different lesion. It produced saltation I-3.

Strain N-3 arose in 1925 as a saltation in a culture of I-2 and has remained stable.

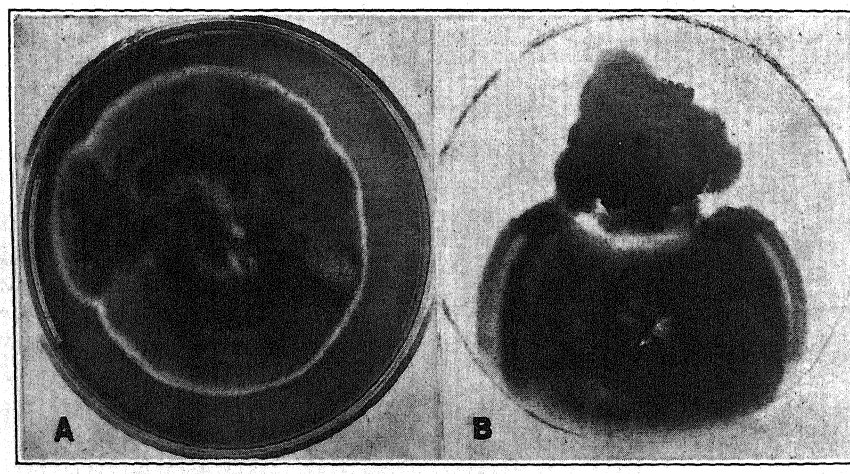


FIG. 1. Typical saltations of *Alternaria solani*. A. A saltation appearing as a darker and faster-growing sector. B. The lower colony differs from the upper (original strain) in that it is slightly chromogenic and grows more rapidly on artificial media.

Typical saltations are shown in figure 1. A is a chromogenic culture of strain I-3 with a saltation appearing as a sector that is darker and faster growing than the main body of mycelium. Often such sectors differ from their parent culture by being nonchromogenic. B is the result of two trans-

fers made thirteen days previously from the same tube culture of strain N-1. The smaller colony is typical of the strain. The other colony differs from the original culture by being slightly chromogenic and producing a faster growth on media.

The saltation history in one related group of cultures is shown in figure 2. It is noted that the chromogenic strain produced both nonchromogenic and intermediate forms similar to those isolated from nature. One strain obtained from this source as a saltation has remained nonchromogenic and is only slightly pathogenic.

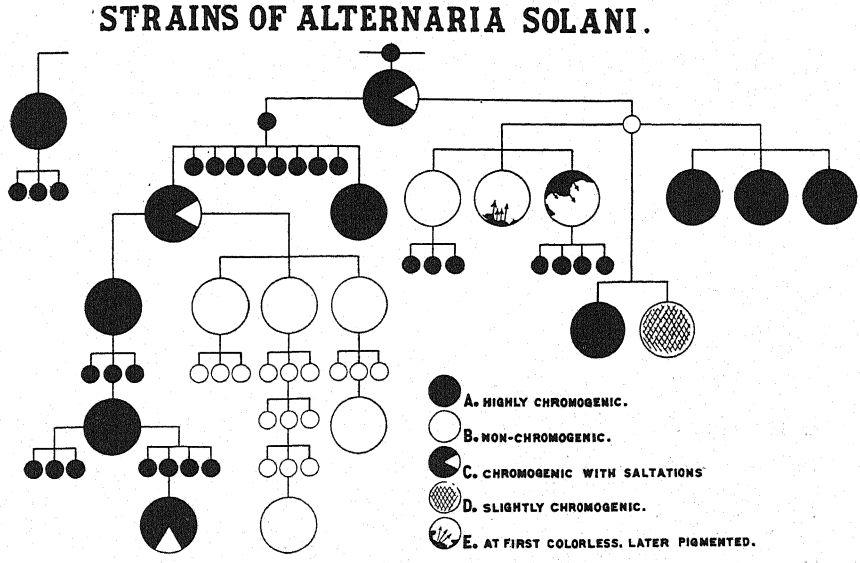


FIG. 2. Diagram showing chromogenicity in progeny originating from a single chromogenic culture of *Alternaria solani*. Small circles indicate test-tube cultures and large circles indicate plate cultures. The latter supply all the saltant sectors but note that transferring either from tube to plate or from plate to tube may be followed by the appearance of a change in chromogenicity.

INDUCING SPORE PRODUCTION IN CULTURE

Spore production on media was found rather difficult to induce and is in need of further study. An examination of numerous cultures grown on potato agar has disclosed only an occasional spore. Cultures grown on prune agar and on corn agar failed to produce spores. Two strains were cultured on sterilized potato plugs, on which spores were more common but were by no means abundant. These spores were deformed in most cases, having swollen cells and crooked or twisted beaks. (Normal spores are depicted in PHYTOPATHOLOGY, Vol. 15, Plate V.) (5.)

As shown by Rands (16) and Kunkel (12), the most successful method of spore production of *Alternaria solani* involves the laceration and desiccation of agar cultures. Rands grew the cultures on petri dishes of hard potato agar and after 10–12 days they were shredded to bits and evenly distributed over the surface of the dishes. The humidity must be controlled for 24 to 48 hours in such a manner as to allow partial desiccation, but not complete drying or hardening of the cut surfaces. Such moisture control is most readily secured by removing the lid and exposing the dish to sunlight in a moist chamber and by atomizing the cultures with sterile water if too dry. If the procedure is successful, spores will develop in great profusion on the cut surfaces of the medium.

The moisture relation between the shredded culture and the surrounding atmosphere is rather critical. When there is enough moisture to condense on the sides of the bell-jar, or in the petri dishes, no spores are produced. The same is true when the culture becomes dry and hard. One can regulate the atmospheric moisture by removing the covers of the petri dishes if the cultures are too wet and by atomizing with water if too dry. Shredded cultures in large test tubes failed to sporulate. This probably was due to the fact that the moisture liberated made an atmosphere too humid for the production of spores.

SPORE PRODUCTION BY DIFFERENT STRAINS

Alternaria solani strains differ greatly in their ability to produce spores in culture. Seven strains, C-1, C-3, N-1, N-2, N-3, I-1, and I-2, representing the different physiological types, were used in the study of spore production and morphology. Many cultures of these strains were subjected to the conditions found generally to be best for the production of spores. Some strains sporulated in great profusion, while others either failed to sporulate or did so only meagerly. Many attempts to produce spores with the latter strains have been made but the results were similar, in that no spores or relatively few appeared. It was found that the strains vary greatly in their ability to fruit on an artificial medium such as potato agar. Strains C-1, C-3, N-2, and I-1 were consistently good spore producers. Strains N-1, N-3, and I-2 have in all cases failed to sporulate freely.

The degree of chromogenicity of a strain is not correlated with its ability to produce spores in culture. Good or poor spore formation was found associated with either chromogenic or nonchromogenic strains. Likewise both chromogenic and nonchromogenic strains are included among those which have failed entirely to form spores on artificial media.

The *Alternaria solani* strains isolated by the writer differ as to the kind of mycelial growth. A black-surface, deep, and compact mycelial mat is

associated with abundant spore production. Strains or cultures possessing a white-surface mycelium, thin and loose in texture, are poor spore formers. There is evidence also that during the process of continued culture some strains have lost their power of forming spores.

BIOMETRY OF SPORE DIMENSIONS OF DIFFERENT STRAINS

The different strains used in the study of spore dimensions were grown on potato agar and made to sporulate in the manner previously described. The medium and environmental factors were made as uniform as possible for all cultures. The writer, however, found it very difficult to control the environment of the mycelium in the culture tubes and differences in the environment may explain the great variation in the mean dimensions found to exist in different cultures of the same strain, to be noted later.

Some strains, such as I-2, produced spores very sparsely and therefore were treated many times before enough spores were produced from which to obtain measurements. Consequently their spores were not all grown at the same time nor were they taken from the same lot of medium. Attempts were made to measure at least 50 spores chosen at random for each population. The spores usually were washed from the cultures with distilled water and mounted immediately on slides for microscopic observation and measurement.

In table 1 are given the mean spore dimensions for cultures of different *Alternaria solani* strains with their constants. A study of table 1 brings

TABLE 1.—*Dimensions of spores from strains of Alternaria solani grown on potato agar*

Strain	Culture Number	Number of spores measured	Length (microns)		Width (microns)	
			Mean and P. E.	S. D. and P. E.	Mean and P. E.	S. D. and P. E.
C-1	1	50	123.80 ± 4.56	47.78 ± 3.22	14.50 ± .200	2.10 ± .142
	2	47	148.19 ± 3.74	39.25 ± 2.65	16.24 ± .387	3.90 ± .274
	3	50	163.40 ± 3.62	37.92 ± 2.56	17.26 ± .393	4.12 ± .278
	4	100[a]	142.70 ± 2.46	36.44 ± 1.74	15.25 ± .163	2.42 ± .115
C-3	1	50	122.00 ± 3.03	31.82 ± 2.15	15.40 ± .227	2.38 ± .160
N-1	1	41	140.36 ± 3.27	31.09 ± 2.31	15.44 ± .237	2.25 ± .167
N-2	1	100[a]	150.40 ± 2.79	41.43 ± 1.97	16.59 ± .169	2.51 ± .119
N-3	1, 2, 3	50	134.00 ± 3.08	32.32 ± 2.18	14.74 ± .187	1.98 ± .133
I-1	1	50	137.40 ± 3.20	33.51 ± 2.26	14.24 ± .166	1.74 ± .117
	2	48	143.90 ± 3.37	34.64 ± 2.38	14.60 ± .202	2.08 ± .142
	3	50	157.00 ± 2.97	31.11 ± 2.10	15.38 ± .190	1.99 ± .134
I-2	1	7[b]	119.30 ± 6.78	26.60 ± 4.79	14.57 ± .405	1.59 ± .028

[a] Preserved in 4-per cent solution of formaldehyde between sporulation and measurement.

[b] From several cultures; many cultures of this strain examined with no spores to be found.

out the fact that the spore dimensions vary greatly for different cultures of the same strain. Culture 1, strain C-1, gave spores that were among the shortest ones measured. Culture 2 from the same strain produced spores having the greatest mean length. Here exists as great a variation as is found by comparing any two distinct strains.

The mean widths of the spores from different strains ranged from 14.24 μ for culture 1, strain I-1, to 17.26 μ for culture 3, strain C-1. The variation between the mean widths of spores from cultures of strain C-1 was nearly as great as the extreme difference between the means of different strains (14.50 ± .200 for culture 1 to 17.26 ± .393 for culture 3 of strain C-1). A greater spore width was generally associated with a greater spore length for the strains measured. In general, the variation of the width was less than that of the length.

No one strain was consistently wider or longer than the others. Apparently, uncontrolled factors caused a greater variation in mean length and width than did a difference in strain. It is probable that some strains are inherently different as to spore dimensions, but with the present methods they can not be differentiated.

GENERAL NATURE OF PIGMENTATION BY ALTERNARIA SOLANI

Unless otherwise specified, "pigmentation" in this paper means the coloration of the agar substratum by products of fungal growth and is to be distinguished from pigmentation of the mycelium which is the subject of most of the literature dealing with the color of fungi *in vitro*.

On potato agar[3] a typical young chromogenic culture at first causes a clear yellow pigmentation which spreads in advance of the mycelial growth of the fungus. This color changes in time to a deep brick red or carmine.[4] It would seem that the *Alternaria solani* strains cultured by Rands were all of a chromogenic type. He also states that "after 7 to 10 generations in pure culture the pigmentation is much diminished and in some cases has been observed to almost disappear" (15, p. 20). However, cultures observed by the writer and his associates in Maine showed marked differences in chromogenicity directly after isolation from potato tubers. About nine-tenths of the several hundred cultures thus obtained were highly chromogenic. The others were either nonchromogenic, some remaining so under all conditions to which they were subjected, or they ranged from nonchromogenicity to marked coloration. Chromogenicity could not be predicted from the appearance of the tuber lesion.

[3] The potato agar used in these studies was made up of 200 grams of pared potato tuber, 20 grams of dextrose, 30 grams of agar, and 1 liter of distilled water. It was slightly acid with a pH value of approximately 6.7–6.8, using brom thymol blue as the indicator.

[4] Color terminology here is based upon Ridgway (17).

Very little is known regarding the chemical nature of the pigment of *Alternaria solani*. When the carmine agar of old chromogenic cultures is shredded and washed with water, a solution of the pigment is obtained which is orange or yellow when made acid and carmine when made alkaline. Therefore the pigment is an indicator. These data also suggest that the fungus increases the alkalinity of potato agar, which will be proved later.

EFFECT OF MEDIUM ON PIGMENTATION

Rands (15, p. 20) reports that *Alternaria solani* produces no coloration when grown on acid casein agar, nutrient gelatin containing dextrose, starch nitrate agar, and cellulose agar. The effect of difference in the kind of medium was brought out when a series of isolations made on prune agar from potato lesions gave in all cases nonchromogenic cultures. Different chromogenic strains of *Alternaria solani* grown on prune agar at different temperatures produced a slight discoloration approaching a light coffee brown or pale xanthine orange, while nonchromogenic forms caused merely a slight murkiness on the agar.

EFFECT OF TEMPERATURE ON PIGMENTATION

During the winter of 1924–1925 various strains were grown at different temperatures to test the influence of this environmental factor upon the degree of pigmentation. The following approximate mean temperatures were secured in dark chambers by the use of an electric device with automatic control together with ice: 5°, 10°, 15°, 20°, 25° and 30° C. The three lowest temperatures were known to fluctuate one or two degrees, varying most from the mean just before and after the process of icing which occurred about once a week.

The highly chromogenic strains continued to be chromogenic in the 20°, 25°, and 30° chambers. Strain C-1 was chromogenic under all conditions except at 5° C. Strains C-2 failed to produce pigment at temperatures below 20° C. The nonchromogenic strains N-1, N-2, and N-3 failed to produce pigmentation in all the cultures observed.

One variable strain, I-1, was slightly chromogenic at 25° and 30° C., producing faint pink or xanthine orange coloration, but at 5° to 20° C. it was nonchromogenic with an occasional trace of pigment as growth progressed. Strains I-2 and I-3 were nonchromogenic at 5° and 10° C. Chromogenic cultures of I-3 became nonchromogenic with age, a phenomenon also noted to occur in other chromogenic cultures.

These studies indicate finally that temperature influences the amount of pigment produced by *Alternaria solani* on potato agar. In general, temperatures below 15° C. may entirely inhibit the production of color. At

approximately 20°, retardation occurs, while at 25° and 30° a maximum amount of color is produced. The maximum rate of growth also took place in the 25° and 30° chambers. The formation of pigment is generally correlated with the rate of growth, although a luxuriant mycelium developed ultimately at the lower temperatures.

Subtransfers were made from the nonpigmented cultures of the chromogenic strains grown at the lower temperatures to test whether the condition was merely temporary due to environment or was permanent due to some change in the mycelium. In no case could it be noted that the ability to produce color had been permanently affected. The mycelium transferred from nonpigmented cultures of normally chromogenic strains consistently produced pigment when grown under higher temperatures. Although these transfers showed that low temperature had no permanent effect on the chromogenicity of a strain of the fungus, low temperature did have a permanent effect on the actual culture exposed. When nonpigmented cultures of chromogenic strains were removed to the chambers with higher temperatures, pigmentation was not produced. This would suggest either that the low-temperature cultures had developed an inhibiting principle in the substratum which prevented the production of the color, or that the pigmentation was a function of rapid growth and, therefore, impossible under any conditions after growth of the substratum had been completed.

EFFECT OF INITIAL HYDROGEN-ION CONCENTRATION ON PIGMENTATION

The variability in chromogenicity in cultures of *Alternaria solani* raised the question as to whether slight differences in acidity of the medium might be responsible for this phenomenon. Accordingly, transfers from vigorously growing cultures of both nonchromogenic and chromogenic strains of the fungus were made to media adjusted to different degrees of acidity and alkalinity.

For these studies strain C-1 and chromogenic cultures of strain I-2 were taken as representatives of the more highly chromogenic types, strain I-1 as representative variable form, and N-3 as a consistently nonchromogenic strain.

A preliminary test was made to determine whether a critical point, at which chromogenicity was affected, could be approximated. Different numbers of drops of N/20 HCl and N/20 NaOH were added to tubes each containing 10 cc. of slightly acid potato agar, following the "drop method" suggested by Hopkins (10, 11). Ten different concentrations were thus obtained ranging from distinctly acid to distinctly basic. The cultures were grown in duplicate at 25° and 30° C. and observations were made at five- to ten-day intervals. A third set of noninoculated tubes, representative

of all degrees of acidity or alkalinity, was kept for a later determination of the pH value.

The distinctly chromogenic strains were not greatly influenced by addition of acid or base. The nonchromogenic strain N-3 failed to produce pigmentation. In the tubes of the two highest acid concentrations, growth was slightly retarded and the pigment formed by the chromogenic strains was slightly subdued and more yellow. The variable strain I-1, on the other hand, was greatly affected by initial acidity and failed to produce pigment at the two highest acid concentrations. The less acid cultures were slightly pink and turned carmine with age. The color change was very striking at this point, the pH value of which was then determined. A set of colorimetric standards was made using various indicators (2, p. 80–81). Noninoculated check tubes, several weeks old, were melted with the indicators present in them and the results showed that the critical point for pigment production was between pH 4.6 and 5.6, within the range of methyl red. It should be emphasized that this critical point refers to the initial hydrogen-ion concentration at the time of inoculation and not at the time when pigmentation is effected. In the interval, as will be shown, the hydrogen-ion concentration is altered by the fungus.

The experiment was repeated and a series of tubes with a greater range of acidity was used. The results were similar to those of the first, with the exception that the variable strain (I-1) was nonchromogenic in all the tubes of media having a greater initial acidity than pH 4.2 and 4.0. An occasional noncolored culture occurred in tubes with a lower initial acidity. This strain was also grown on media made acid with N/5 instead of N/20 HCl, thus securing a still wider range of acidity. In this series noncolored cultures were consistently secured where the initial acidity of the medium was more than pH 4.2.

Similar sets of cultures of the same variable strain were made basic by adding N/5 NaOH to tubes containing 10 cc. of slightly acid agar. The basic cultures produced intense carmine pigmentation in most cases. The pigmentation faded when the NaOH was added in excess of 9 drops per 10 cc. The color of the less basic cultures approached that produced normally by chromogenic strains. As stated previously, a solution of the pigment from red cultures also became distinctly carmine when made basic.

These data are not in accordance with the observations made by Rands (15, p. 20), who apparently had still different strains of the fungus. He found that the fungus produced a red to deep red color on distinctly acid media (+20 Fuller's scale), a yellow color on slightly acid cultures and practically no pigment in alkaline cultures. In view of the observations already made, the writer believes that with more extensive study other

strains can be obtained which will differ greatly in their reactions to the hydrogen-ion factor.

EFFECT OF LIGHT ON PIGMENTATION

Cultures of the different strains were grown in the greenhouse in the presence of diffuse light and five successive attempts under these conditions did not affect the amount of pigmentation produced by a chromogenic strain. Nonchromogenic strains likewise were not affected visibly. Under greenhouse conditions the growth of some cultures was retarded and the pigment was made slightly dull.

Strain I-1, however, was influenced by light, and cultures grown in the greenhouse were constantly more pigmented than those in the dark chambers. Instead of being slightly pink or nonchromogenic they were yellow or xanthine orange. To verify further the effect of light on this variable strain, a more careful test was performed. Uniform tubes of agar were inoculated with a single-spore strain (from strain I-1) and lots of three tubes each were placed in the greenhouse and in the 20° dark chamber. The cultures in the dark failed to produce color, while those grown in diffuse light developed a reddening approximating the intensity observed in cultures of the more constantly red strains. This experiment was repeated several times with similar results.

Test-tubes containing the strain just previously referred to were wrapped in photographers' black paper and grown in the greenhouse along with similar tubes not wrapped. These two sets of cultures were compared with a third set of unwrapped cultures kept in the dark chamber. The cultures darkened by paper and those in the dark chamber produced a slight pinkish discoloration in contrast to the reddish brown and xanthine orange of the tubes kept in diffuse light.

EFFECT OF WATER CONTENT OF AGAR ON PIGMENTATION

Desiccation of the agar appeared not to influence the amount of pigmentation produced by the variable strain I-1. Tubes of neutral agar were desiccated by storage in a warm, dry place. To some of these tubes water was added. After being melted and reslanted both sets of tubes were inoculated and grown in the greenhouse either in the presence or in the absence of light. Similar cultures on desiccated and rewatered agar also were grown in the 20° C. dark chamber in a series parallel with that in the greenhouse. Desiccation in this test did not affect the degree of pigmentation. The cultures in both desiccated and moist media were only slightly colored in the chamber and in the tubes darkened by the black paper. In contrast, all of those kept in diffuse light developed the xanthine orange pigmentation.

INFLUENCE OF ALTERNARIA SOLANI ON THE HYDROGEN-ION CONCENTRATION OF POTATO AGAR

As stated in the foregoing, a typical culture produces an intense red color in potato agar. By treating a shredded culture with water or grain alcohol, this pigment is readily removed. As observed above, it was found that the pigment has the properties of an indicator, and is dull orange or amber in acid solution and deep carmine or wine color in basic solution.

In view of this phenomenon and the fact that cultures usually become more highly pigmented with age, an attempt was made to determine the influence of the fungus on the hydrogen-ion concentration of the medium. A nonchromogenic strain, N-3, was grown on potato agar in the presence of different indicators. In the first trial brom phenol blue, methyl red, brom thymol blue, and phenol red were used (2, p. 80–81). The indicator was added to the melted agar and N/5 HCl or NaOH added until series of tubes were obtained having the proper pH ranges for the respective indicators. The tubes were then slanted and inoculated. By growing the fungus in the presence of these indicators and observing the color changes, it was expected that a clue could be obtained as to the amount of acidity or alkalinity produced in the medium.

The growth of the fungus destroyed the efficiency of brom phenol blue, brom thymol blue, and methyl red. That is, the media lost the distinctive colors produced by these indicators and became non-pigmented. Phenol red, however, was not inactivated. The medium originally was slightly acid, having a pH value of approximately 6.6 to 6.8. In the presence of phenol red the medium supporting the growth of the strain in question became distinctly red in all cases, demonstrating that alkalinity had been produced beyond about pH 8.0. The experiment was repeated with the same nonchromogenic strain, which again produced an alkaline reaction as indicated by the phenol red.

The chromogenic strains were found to be unsuitable for study by this method because the pigment they produce is intense enough to mask the colors of the indicators. Therefore the culture-washing method was again resorted to with cultures of several nonchromogenic and chromogenic strains. In dilutions thus obtained the natural indicator (*i.e.*, the pigment produced by the fungus) was not concentrated enough to mask the phenol red, which gave a color reaction at the basic end of its range. Phenolphthalein, however, gave an acid color reaction showing the pH value of the solution to be less than 8.3–8.4.

The cultures of the preceding test were from 14 to 60 days old. It was thought probable that after a longer period of growth the pH value might be higher. Therefore cultures were grown for several months in the presence of phenolphthalein. In that time the alkalinity produced was not

great enough to bring about the red color in the indicator. The addition then of NaOH gave the basic reaction and showed that the indicator was still present and active.

THE PIGMENT AS AN INDICATOR

The above data throw some light on the nature of the chromogenicity of the different strains. The degree of color is dependent on two factors, namely, the amount of natural indicator and the alkalinity produced by the organism in the medium. The nonchromogenic strains increase the alkalinity like the other strains but do not produce this natural indicator.

The variable strain I-1 is slower to produce pigmentation but in old cultures sufficient amounts of natural indicator and alkalinity may occur to give a decided carmine hue. This may explain the reason why cultures, at first thought to be nonchromogenic, later become decidedly colored. Young 3- to 6-day-old cultures of the chromogenic strains are yellow because the medium is not yet alkaline enough to change the pigment to the carmine color. Plate cultures, carmine in color, have been made yellow by the addition of a weak acid. In such cases the fungus produced in a few days enough basic substances to neutralize the acid and the carmine color again appeared.

An attempt was made to determine the active pH range of the indicator produced by the organism. Water and alcoholic extracts of the indicator obtained from chromogenic cultures were tested for their color reaction in standard pH solutions for the range from 3 to 7.8. This range was found to be too acid to change the pigment of the fungus from yellow to red. This would indicate that a greater alkalinity than pH 7.8 is necessary to produce the red color of this fungous product. This confirms the preceding conclusions as to the pH value of old cultures.

PRODUCTION OF A TOXIC SUBSTANCE BY NONCHROMOGENIC STRAINS

There is evidence that the nonchromogenic strains produce a substance or substances toxic enough to inhibit both the growth and the pigmentation of the chromogenic strains. It was noted that a chromogenic strain planted in an agar plate on which a nonchromogenic strain was already growing often failed to grow. The mycelial mat then was removed from nonchromogenic cultures and the remaining agar melted and reslanted. This medium would not support the growth of the chromogenic strain C-1. The experiment was not extended to include the other strains.

When two chromogenic strains are grown simultaneously in the same plate of potato agar, the pigment readily diffuses throughout the medium. However, when the nonchromogenic strain N-3 was planted in close proximity to the chromogenic C-1 strain in the same plate, the pigmentation

of the agar was retarded in the region about the nonchromogenic organism. The chromogenic strain continued the formation of pigment in the part of the plate remote from the growth of the nonchromogenic strain. That the production of the natural indicator and not that of alkalinity is inhibited is evidenced by the alkalinity of nonchromogenic cultures.

PATHOGENICITY OF STRAINS ON LEAVES AND TUBERS

A study of the relative pathogenicity of the strains of *Alternaria solani* revealed that a great difference exists in their ability to infect potato foliage. In preliminary inoculations, however, the tested strains were equally virulent when tested on potato leaves. Two chromogenic strains, C-1 and C-4, and two nonchromogenic strains, N-1 and N-2, were used in this initial comparison. The four strains used in these tests were all pathogenic and caused rapid collapse when inoculated onto potato plants.

Clinton and McCormick (3) found moist petri dishes convenient chambers for the inoculation of leaves with certain rust fungi. The writer adopted a modification of their method for the study of the virulence of *Alternaria solani* strains. Damp chambers about 16 cm. in diameter were used for potato-leaf inoculations. Heavy blotting paper was cut to fit the bottom of each container. The blotter was moistened with water and marked with an indelible pencil into four equal sectors. Susceptibility depends somewhat on the age of the leaves. Accordingly, four similar leaflets from the same leaf, taken from a plant of the Green Mountain variety grown in the greenhouse, were placed in the four sectors to be respectively inoculated with four strains of the fungus. A fifth leaflet from the same leaf was placed in the middle, at the junction of the sectors, as a noninoculated control. A second leaf was used in the same way in each chamber. In each series of inoculations, strain C-1 was compared with three other strains. Each comparison was made on 30 leaves in 15 damp chambers.

When the mycelium had grown sufficiently to cover most of the surface of the medium in the tubes, inoculations were made by placing uniform bits of the mycelium on the ends of the leaflets. The rapidity with which the fungus destroys potato leaves depends to some extent on the prevailing temperature and moisture. Moisture was applied by means of an atomizer whenever the inoculum showed evidence of becoming too dry. The culture chambers were placed in diffuse sunlight in the greenhouse. Total darkness in controlled temperature chambers caused chlorosis and rapid collapse of the inoculated leaflets. Direct sunlight often resulted in a withering of the leaves and in desiccation of the inoculum.

The degree of pathogenicity was estimated by measuring the diameter of the brown, obviously infected area associated with the inoculum on the leaf surface, 5 or 6 days after inoculation. The diameter of the two spots

on each leaflet were averaged and the data analyzed by Student's method (13, 14). The means and the probability or odds that the difference is significant and not due to mere chance are given in table 2 for the two series of comparisons that were made.

The leaf inoculations with the various strains showed that a great variation in pathogenicity may exist. Strain C-1 which was used as the control was considerably and significantly more pathogenic than strains N-1, N-3, and I-1, while, according to the odds, it was in the same class with strains C-5 (dark) and N-2 and was not much more pathogenic than strain C-2. The last comparison is interesting because strain C-5 originated as a saltation from strain C-2, the saltation differing from its parent by possessing a darker colored mycelium.

TABLE 2.—*Comparison of strains of Alternaria solani as to pathogenicity on potato leaves in vitro*

Series[a]	Strains compared, with mean diameter of lesion (in mm.)		Significance of difference expressed in odds[b]
1	C–1 (17.43)	C–2 (14.17)	About 76.5
	C–1	C–5 (17.67)	Less than 2.37
	C–1	N–1 (3.60)	Over 9,999
	C–2 (14.17)	C–5 (17.67)	About 76.5
	C–2	N–1 (3.60)	Over 9,999
	C–5 (17.67)	N–1	Over 9,999
2	C–1 (11.87)	N–2 (11.70)	About 8.82
	C–1	N–3 (4.70)	Over 9,999
	C–1	I –1 (3.67)	Over 9,999
	N–2 (11.70)	N–3 (4.70)	Over 9,999
	N–2	I –1 (3.67)	Over 9,999
	N–3 (4.70)	I –1	About 5.90

[a] Each series consisted of 30 comparisons; see text, p. 545.
[b] Odds should be 30 or over to be significant.

The variation in the ability of different strains to infect foliage raised the question as to whether the strains might also show differences in virulence when inoculated into potato tubers. The strains used for the leaf-infection studies were grown on steam-sterilized wheat kernels. The wheat grains covered with the fungus were placed on freshly harvested Spaulding Rose tubers and incubated in moist chambers. Each tuber was inoculated with 10 kernels.

Strain I-1 was consistently and undoubtedly pathogenic in these tests. Strain I-3, originating as a saltant, was very weakly parasitic, causing only a few small and shallow lesions. All the other strains failed to produce spotting and lesions. It is significant that in a repetition of the test four substrains originating from single-spore colonies of strain I-1 were all pathogenic. The pathogenic strains were reisolated from the tuber lesions,

compared with the original cultures, and found to be identical with them.

It has seemed possible that strains of *Alternaria solani* might lose their virulence during continuous culturing on artificial media. However, strain I-1 has been cultured since 1922 and has not lost its pathogenicity to tubers. This would seem to show that loss of pathogenicity does not necessarily occur on artificial media within four years.

Strain I-1, though most pathogenic on tubers, was among the strains less pathogenic to potato leaves, while a reverse relation holds for some of the other strains.

These comparisons as to relative virulence indicate clearly that a great difference may exist between certain strains in their ability to attack potato foliage. Whether these differences exist in the field is an interesting question not yet answered. How often such differences arise through saltation, both in culture and in nature, is a problem awaiting attention. With a production of new strains in nature, the problem of early blight control might become more complicated than it seems to be at present. Various strains may be found to differ as to their pathogenicity to the varieties of the potato or genera and families of host plants. It may be proved eventually that under natural conditions some strains are virulent on potato foliage but unable to infect tubers.

SUMMARY

(1) *Alternaria solani* comprises strains which can be differentiated on the basis of the production of spores, the formation of pigment in potato agar, the rapidity of growth, the appearance of the mycelium in culture, the formation of lesions on detached leaves and tubers and frequency of saltations.

(2) Spore production required particular conditions. The strains varied as to the abundance of spore production under apparently similar conditions. When spores were produced their mean dimensions varied as much in different cultures of the same strain as in cultures of different strains, so that spore measurements did not serve to differentiate strains.

(3) The great majority of the strains isolated from lesions on potato tubers were chromogenic on potato agar, turning it red. Other strains were nonchromogenic, and some were intermediate or variable as to chromogenicity on potato agar. The pigment is an indicator which in aqueous or alcoholic solution turns from yellow (acid color) to deep red or carmine (alkaline color) at a pH value of about 7.8. Temperature influences the amount of pigment produced by certain more or less chromogenic strains, the temperature at the higher range of growth being optimum. In one intermediate strain the chromogenicity was inhibited by a lower initial hydrogen-ion concentration and by the absence of light but was not affected

by desiccation of the medium. Chromogenic strains do not redden prune agar. All strains increased the pH value of potato agar from about 6.6 to 8.3, as indicated by tests of aqueous and alcoholic washings of the agar. Nonchromogenic strains made potato agar toxic to chromogenic strains and prevented pigment formation by the latter.

(4) Inoculation by placing sterile mycelium on detached potato leaflets, or tubers, in damp chambers revealed highly significant differences in the pathogenicity of certain strains.

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RELATION OF SPECIES OF PYTHIUM TO THE ROOT-ROT DISEASE OF SUGAR CANE

C. W. Edgerton, E. C. Tims and P. J. Mills

The trouble of sugar cane known variously as root rot, root disease, root-rot complex, and growth-failure complex has been known and studied in sugar-cane countries for a long period. The most prominent symptoms include the failure of the plants to grow satisfactorily, the light yellow color of the leaves, and the decay of the root system. The poor growth, always characteristic of root rot, results from the failure of the root system to function satisfactorily. In severe cases, the roots may be so extensively rotted that a whole stool may be pulled from the ground with very little difficulty. In less severe cases, the roots show only rotted ends and few laterals.

Since many factors may influence the development and functioning of the root system, it is not reasonable to presume that the root-failure trouble may always be due to a single cause or be produced by the same factors in every country. As a matter of fact, various workers at one time or another have suggested many causes for the root rot. Among others, these include the chemical and physical condition of the soil, the presence of toxins in the soil, and the attack by certain soil-inhabiting animals and fungi. While the present article treats only of the relation of certain fungi to the root rot as it occurs in Louisiana, it must be remembered that there are also a number of other important contributing factors.

A study of the literature relating to the root rot of sugar cane shows that a number of different fungi have been associated with the disease. Of these, those given most attention at one time or another include species of Marasmius, Rhizoctonia, and Pythium. While none of these species have been entirely eliminated from the problem, most of the work now being carried on in different sugar countries is with species of Pythium. Inoculation experiments with most of the other fungi have not given consistent and satisfactory results.

Fungi of the Pythium type have been reported at various times on cane roots, but the first satisfactory evidence that any of these had anything to do with the root disease was presented by Carpenter (3) in 1919. He found coenocytic mycelium and oospores in roots of cane suffering from the Lahaina or root rot and associated this mycelium with the flabby, rotten root tips characteristic of root rot. He also isolated the fungus and was able to obtain typical rotting of roots by pure-culture inoculations. (4, 5).

In 1924, Bourne (1) reported a fungus on cane roots in Porto Rico, which he considered very similar to the Pythium described by Carpenter

in Hawaii. Bourne, however, reported the occurrence of numerous oospores in his cultures, this being different from the observations of some of the other investigators.

The relation of Pythium fungi to the root disease, as it occurs in Louisiana, has been treated in articles by Edgerton and his coworkers (8, 9, 10) and by Tims and Mills (13). In these articles, it was stated that numerous fungi were found associated with the decay of cane roots but that inoculation tests were unsuccessful with all except certain species of Rhizoctonia and some Pythium forms. As the Rhizoctonia forms were thought to be of only minor importance, most attention was paid to the Pythiums which were found associated with decaying cane roots.

In the present paper are given the results obtained at the Louisiana Agricultural Experiment Station from more than three years' work on one phase of the root rot, *i.e.*, the relation of certain species of Pythium.

RELATION OF PYTHIUM TO ROOT-ROT DISEASE

The roots of cane plants showing symptoms of root rot are always affected to a greater or less degree. Many of the main roots, both primary and secondary, are short and stubby, due to a decay of the tip portions. As the root tips decay, branches attempt to form back of the diseased portions, but these also usually become infected. Consequently, instead of the long straight roots, characteristic of healthy plants, roots of affected plants are short, crooked, and branched. Furthermore, the small fibrous roots, usually abundant on healthy plants, are few on diseased plants. They seem to rot off as rapidly as they form. In all cases, the young, most rapidly developing portions of the roots are the parts most subject to decay. The affected root tips are grey to brownish in color and are soft and flabby. In the diseased roots, coenocytic mycelium, and oospores characteristic of the genus Pythium, commonly occur.

A root-rot condition similar to that occurring in the field has been produced many times by inoculating young plants in sterilized soil with pure cultures of a Pythium, which occurs commonly on decaying roots in the field. For these tests, soil in six-inch pots was sterilized in an autoclave. After the soil had cooled, about one to two inches was removed from the top and the inoculum which consisted of an agar plate culture of the Pythium was mixed with the soil remaining in the pot. Then seed pieces containing good buds were placed in the pots and covered with the soil which had been removed. As the roots developed, they immediately came in contact with the Pythium-infested soil.

Hundreds of inoculations have been made by this method and in all cases the results have been consistent. As soon as the roots began to form, they became infected with the Pythium and showed the typical soft, flabby

rot characteristic of the disease in the field. Usually, very few roots developed and consequently the young plants growing in the inoculated soil made a very poor growth. In figure 1, the plants from a single inoculation

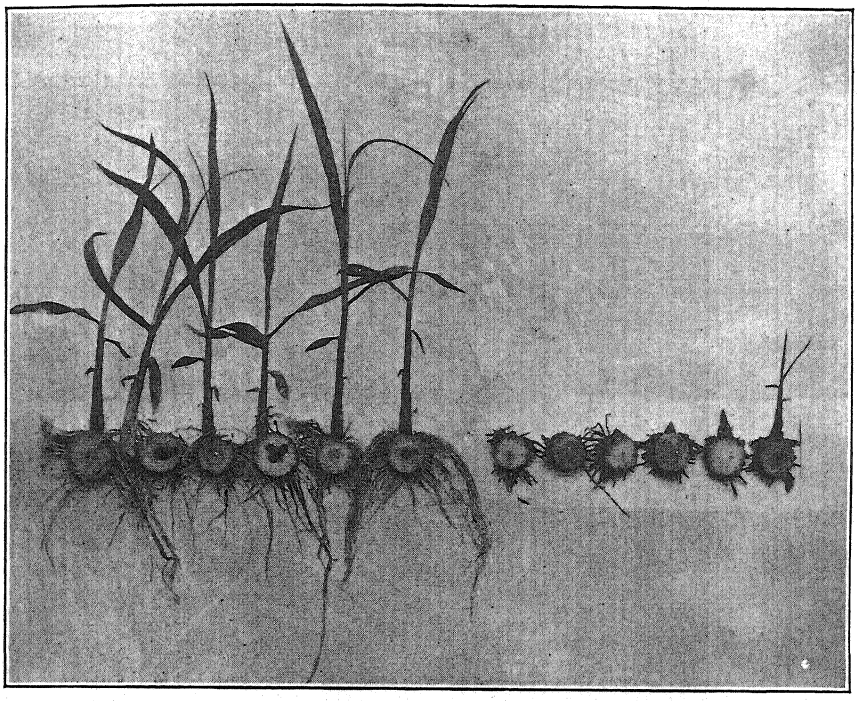


FIG. 1.—Effect of Pythium on young cane plants in sterilized soil. Plants on right, inoculated; those on left, not inoculated. Growth from February 9 to March 9, 1927.

test are shown. The plants on the left were not inoculated while those on the right were inoculated with a Pythium culture.

Inoculation tests have been made also during every month in the year. In many of the sugar-producing countries, it is recognized that the rotting of cane roots is more severe during the cooler, wetter seasons. In Louisiana, root rot is always more serious in seasons following winters of heavy rainfall. In the inoculation tests, the roots of cane have been rotted by the Pythium in both winter and summer, but during the warmer weather, the plants have been able to make a fairly satisfactory growth in spite of it.

Inoculation tests have also been made on all of the common cane varieties, including the resistant P.O.J. canes. The roots of all of the varieties, even of those which show a marked resistance to the disease in the field, have been readily attacked by the Pythium.

These inoculation tests have shown that under certain conditions, some Pythium species are capable of rotting the roots of cane and of producing a rot similar to that commonly seen in the field.

PLANTS AFFECTED BY PYTHIUM

As reported in previous papers (10, 13), the species of Pythium which attack cane also attack certain other members of the grass family but, as far as is known at present, do not attack plants outside this family. Corn, sorghum, and wheat are very susceptible and oats (Texas Red Rust Proof) to a slightly less degree. (Figs. 2 and 3). In the numerous inoculation ex-

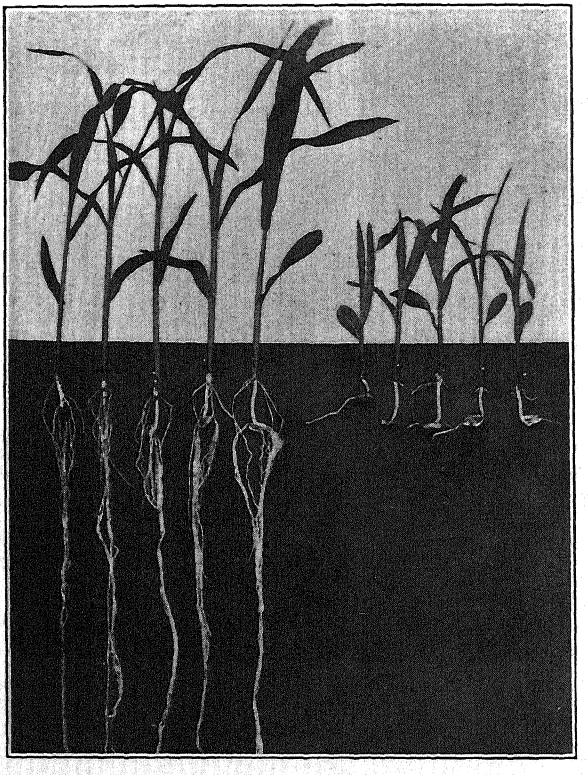


FIG. 2.—Effect of sugar-cane Pythium on young corn plants in sterilized soil. Plants on right inoculated at planting time; those on left were not inoculated. Growth from June 6 to June 17, 1927.

periments carried on in sterile soil, the roots of these plants have been affected in a manner very similar to those of cane and the plants have made a very poor growth. Such legumes as soybeans and cowpeas do not seem to be injured by the cane Pythium. It is interesting to note, however, that

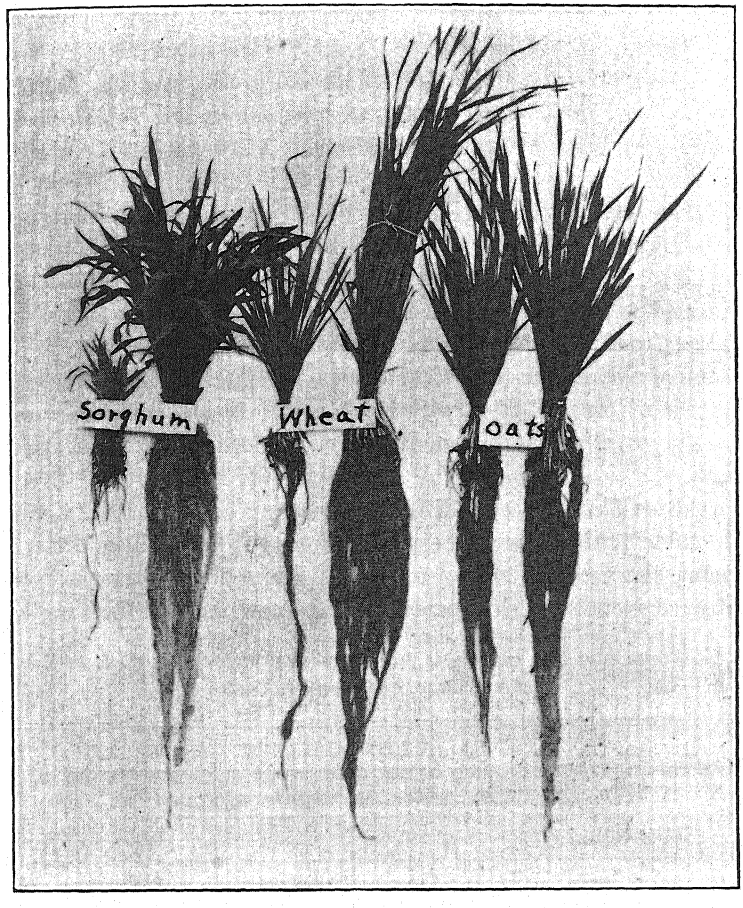


FIG. 3.—Effect of sugar-cane Pythium on sorghum, wheat, and oats in sterilized soil. In each group, the plants on the left were inoculated at planting time; those on the right were not inoculated. Growth from April 19 to May 4, 1928.

there are Pythium parasites of legumes in Louisiana but these do not attack members of the grass family.

The relation of Pythium to the rotting of corn roots has also been mentioned in recent years by Valleau (14), Branstetter (2), Johann (12), and Drechsler (7). These investigators noticed Pythium on the roots of corn and some of them isolated the fungus. Branstetter, in 1927, stated that the fungus found in Missouri was very similar to the Pythium reported by Carpenter in Hawaii.

FUNGI ON CANE ROOTS

In order to determine what fungi are present on cane roots and also the relative abundance of each, a number of surveys have been made over the

cane belt of Louisiana at different times during the past four years. In each field examined, a composite root sample, consisting of more than fifty roots and taken from several different stools of cane, was obtained. These roots were placed in soil-sample cans with sufficient soil to keep them from drying and were brought to the laboratory. After washing in water, small sections, from 5 to 10 mm. in length, were cut from 50 different diseased roots. These were then dipped in a 1–1000 corrosive-sublimate solution, washed in sterile water, and transferred to petri dishes containing bean-pod agar. The plates were incubated at 25° C. to 30° C. for two to eight days and examined. The roots were usually cultured within 48 to 72 hours after being taken from the field.

In some of the earlier reported surveys (9, 13) some of the agar used in the culture work was acidified with lactic acid. As the acid had a tendency to retard the growth of certain of the Pythium forms, it was not used in the later work.

The relative abundance of different fungi on cane roots in Louisiana is shown in table 1, this giving the results obtained from three different surveys during the growing season of 1927. From these surveys, 7890 roots were cultured from 263 fields located in different parts of the cane belt.

TABLE 1.—*Fungi found on cane roots from surveys over the sugar belt of Louisiana in April, June, and August, 1927*

	April, 1927	June, 1927	August, 1927	Total
No. of fields	52	51	160	263
No. of roots cultured	1560	1530	4800	7890
Pythium sp.	273	292	915	1480
Rhizoctonia sp.	217	130	468	815
Marasmius plicatus	16	1	18	35
Trichoderma sp.	300	270	670	1240
Cunninghamella sp.	31	65	196	292
Cephalosporium sp.	45	38	102	185
Aspergillus sp.	36	45	196	277
Fusarium sp.	78	96	171	345
Penicillium sp.	36	28	215	279
Rhizopus sp.	69	69	221	359
Acrothecium sp.	22	53	164	239
Alternaria sp.	26	33	120	179
Phoma sp.	18	16	114	148
Mucor sp.	106	96	272	474
Sclerotium (pink)	7	14	58	79
Sclerotium (black)	7	26	86	119
Oospora sp.	8	15	73	96
Other fungi	107	230	452	789
Free of fungi	367	160	488	1015

An examination of the table shows that a number of different fungi occur quite abundantly on cane roots. Many of these are known to be only saprophytic and have little, if any, bearing on the root-rot problem. Of those known to be parasitic to a greater or less degree, the forms of Pythium were most abundant. Of the 7890 roots cultured, forms of Pythium were observed on 1480. Undoubtedly, many more of the roots were infected with Pythium but growth in the petri dishes was obscured by fast-growing saprophytes. The table shows that forms of Pythium are widely distributed and occur in abundance in the Louisiana cane soils.

FREQUENCY OF PYTHIUMS ON CANE VARIETIES

Certain varieties of cane are known to be very tolerant to the root rot. In Louisiana, varieties like P.O.J. 213 and P.O.J. 36 will make a fairly satisfactory growth under root-rot conditions that will practically stop growth in varieties like Purple and D 74. On this account, it is important to know the occurrence of Pythium on the roots of the different varieties.

In the surveys made in 1927, root samples were taken from a number of the more common and interesting varieties in the different sections of the State. The occurrence of Pythium on the roots of the different varieties is given in table 2. The table shows the percentage of the roots on which Pythium was observed. In each case, fifty roots were cultured from each field.

TABLE 2.—*Percentage of roots of different cane varieties from which species of Pythium were obtained in culture. 1927*

Variety	April, 1927		June, 1927		August, 1927	
	No. of fields	Pythium percentage	No. of fields	Pythium percentage	No. of fields	Pythium percentage
Purple	6	6.1	11	21.6	41	19.8
Striped	6	16.1	4	22.5	4	11.6
D 74	7	8.5	13	17.6	29	21.6
D 95					2	16.6
P.O.J. 36	1	40.0	2	26.6	8	15.0
P.O.J. 213	3	23.3	2	30.0	14	24.5
P.O.J. 228	1	16.6				
P.O.J. 234	18	22.4	14	15.0	27	18.5
P.O.J. 979	2	36.6	2	28.3	8	16.6
L–511	1	30.0			1	10.0
Uba	2	16.6			2	10.0
Cayana 10			3	11.1	2	1.6
Crystallina	1	33.3				

An examination of the table shows that the roots of all of the varieties carried a high percentage of Pythium infection. While there are some variations in the table, these probably are due to the relative amount of Pythium infestation in the different fields rather than the susceptibility of the different varieties. It would seem that Pythiums are as frequent and as abundant on the varieties tolerant to root rot as on the susceptible varieties.

PARASITISM OF DIFFERENT PYTHIUMS

In the several surveys made previous to 1928, no attempt had been made to separate the different types of Pythium found on cane roots. Differences between various cultures had been observed, and also by inoculation experiments it had been determined that certain of the cultures, although isolated from cane, were not parasitic on cane roots.

To determine the distribution and relative abundance of the parasitic forms, a survey was made during January and February, 1928. A composite sample of 50 roots was collected from each field and cultured as in the previous surveys. Pure cultures were obtained of as many of the Pythiums as possible. While some of the Pythiums were lost through contamination of the original plates with fast-growing saprophytes, enough were isolated to give the desired information.

To determine the pathogenicity of the cultures, short-cut methods were devised. To test cultures on cane roots in pots in the greenhouse takes about thirty or more days for satisfactory results. From previous investigations involving many cultures, it had been found that those cultures parasitic on cane are also parasitic on corn. As an inoculation test can be run with corn in about ten days, it seemed just as satisfactory to use this plant to test the parasitism of the large number of Pythium cultures. The effect of different cultures of Phythium on corn is shown in figure 4.

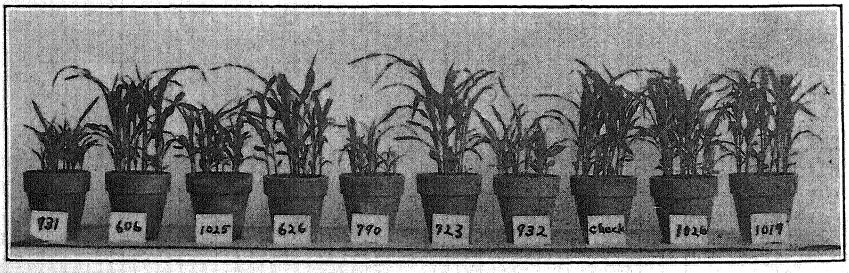


FIG. 4.—Effect of different cultures of Pythium on corn. Cultures 931, 1025, 932, 1026, isolated from cane rots; 606 from spinach; 626 from rhubarb; 790 from corn (received from Miss Johann); 723 from orange; 1019 from cotton.

A still shorter method also was devised which at the present time is being used almost exclusively for parasitism tests and may be found useful to others working with Pythiums. Corn kernels were sterilized in a mixture of corrosive sublimate (1–1000) in 50 per cent alcohol, according to the method used by Chen (6), and then placed in sterile petri dishes containing moistened filter paper. After the hypocotyls had reached a length of two to three centimeters, they were ready for inoculation. Figure 5, A, shows a petri dish containing the germinating kernels as they were used

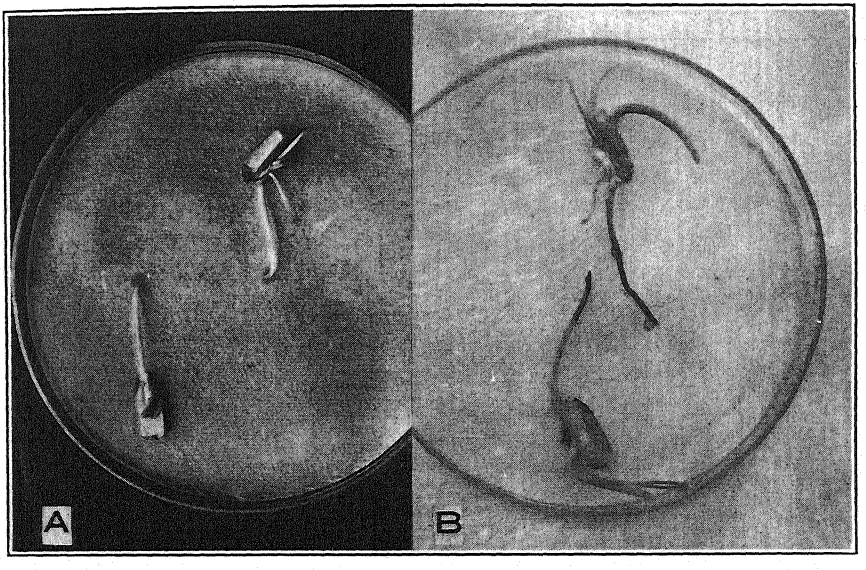


FIG. 5.—Rot produced by a parasitic Pythium on corn roots in petri dishes.
A. Condition of roots at time of inoculation.
B. Condition 48 hours after inoculation.

for inoculation. Sections of agar, 5 to 10 mm. square, from a young, vigorously growing plate culture were placed upside-down on the root tips. The roots were incubated at 25° C. for 48 hours and examined.

In the petri-dish tests, some cultures caused within 24 to 48 hours a complete collapse and decay of the part of the root in contact with the agar. (Fig. 5, B.) These were the same cultures that severely decayed the roots of corn and cane in pot experiments. A few cultures produced only a slight decay of the cortical cells, while others did not seem to affect the roots at all.

In testing the parasitism of the cultures obtained from the survey, both the pot method with corn and the petri-dish method were used. The results from the survey and from these tests are given in table 3.

TABLE 3.—*Occurrence and parasitism of species of Pythium from survey made in January and February, 1928*

Variety	Parish	Occurrence		Parasitism, pot method		Parasitism, petri-dish method	
		Number roots cultured	Number with Pythium	Number cultures tested	Number parasitic	Number cultures tested	Number parasitic
P.O.J. 234	St. John	50	28	25	5	24	6
P.O.J. 36	St. John	50	22	19	17	19	16
P.O.J. 213	St. John	100	36	29	13	28	9
P.O.J. 213	Lafourche	100	32	29	29	27	27
P.O.J. 36	Lafourche	50	38	37	23	36	23
P.O.J. 213	Assumption	100	50	46	34	45	36
P.O.J. 228	Assumption	50	32	21	20	21	20
D 74	Iberville	50	30	20	17	19	17
P.O.J. 234	Iberville	50	12	12	11	11	10
Purple	Iberville	50	32	27	20	26	19
P.O.J. 36	Iberville	50	32	23	23	23	23
P.O.J. 213	Iberville	50	26	22	13	23	13
P.O.J. 234	Iberville	150	103	70	64	70	64
P.O.J. 213	Iberville	50	43	37	35	37	33
P.O.J. 826	W. Baton Rouge	50	23	9	9	9	9
P.O.J. 213	W. Baton Rouge	100	30	22	21	22	21
P.O.J. 36	W. Baton Rouge	50	16	11	8	11	8
P.O.J. 36	Terrebonne	100	38	32	31	32	31
P.O.J. 213	Terrebonne	50	32	27	21	24	19
P.O.J. 213	St. Mary	50	20	20	14	19	13
P.O.J. 213	St. Mary	50	32	19	18	19	18
Purple	St. Mary	50	28	14	14	14	13
P.O.J. 234	St. Mary	50	28	22	22	22	20
P.O.J. 234	St. Martin	100	68	37	33	36	31
P.O.J. 213	St. Martin	50	20	12	10	12	9
P.O.J. 36	St. Martin	50	22	20	17	17	16
P.O.J. 234	St. Martin	50	28	23	21	23	21
P.O.J. 213	Lafayette	50	24	22	20	22	20
P.O.J. 234	Lafayette	50	18	7	5	7	2
P.O.J. 36	Lafayette	50	24	19	17	18	12
Total		1900	967	733	605 (82%)	716	579 (80%)

In the survey, which covered the main portion of the cane belt, 1900 roots were collected and cultured. The percentage observed infected with Pythium varied from 24 to 86. In the inoculation experiments, 733 cultures were tested by the pot method and 715 by the petri-dish method. The results obtained by the two methods agree very closely. By the pot method 82 per cent were found parasitic and 80 per cent by the petri-dish method.

The results obtained from this survey and the inoculation tests show that forms of Pythium capable of attacking the roots of cane and corn are widespread over the cane belt of Louisiana.

TYPES OF PYTHIUM OBSERVED

Among the large number of Pythium and Pythium-like cultures isolated from sugar-cane roots, there have been found a number of different types. Some of these have been studied to a certain extent but no attempt has as yet been made to identify them. While it is very probable that some of them are undescribed species, there has been no opportunity to compare them with accurately determined cultures.

The cultures isolated from cane roots differ not only in their ability to attack cane roots, but also in their morphological characters. As has been noted on a previous page, from a parasitism standpoint, some cultures are extremely virulent on roots of cane and corn, others are mildly parasitic, while still others have practically no effect on living root tissues of these plants. From a morphological standpoint, the cultures differ in regard to the abundance of oospores and conidia formed in culture media, the size of these fruiting bodies, the markings on the surface of the oospores, and the characters of the mycelial threads.

Of the many Pythium types which have been isolated possibly four are worthy of mention, either because of their frequency on cane roots or their parasitic characteristics. It is possible that some of these may represent groups of species rather than single ones. These are briefly described.

1. Of the Pythiums isolated from cane roots, but later found to be nonparasitic to cane and corn, one in particular was common. This form produces smooth oospores, 23–30 μ in diameter, in great abundance on almost any of the common culture media. While not parasitic to cane or corn, some of the cultures, at least, were parasitic on soybeans and cowpea roots. While these nonparasitic forms can have no relation to the root disease, it is possible for the oospores to develop and later be found in dead cane roots. Consequently, the presence of oospores in corn and cane roots does not necessarily mean that parasitic forms of Pythium are present.

2. Another type of Pythium, characterized by abundant production of smooth oospores, but differing from the above form in being moderately

parasitic on cane roots, has been obtained occasionally in pure culture. This Pythium produces oospores readily in pure culture. Several antheridia attached to a single oospore have been frequently noticed in cultures of Pythium of this type. How important this fungus is in the root-rot problem has not been determined.

3. A type of Pythium characterized by spiny oospores has also been observed occasionally. The oospores occur abundantly in the tissues of the roots, including the central stele. A similar fungus has recently been reported by Carpenter in Hawaii. It is not found sufficiently abundant in Louisiana to be important.

4. The Pythium form isolated in Louisiana, which is the most actively parasitic to cane and corn roots and apparently the most important in the root-rot problem, does not produce fruiting structures of any kind readily on ordinary culture media. Oospores have been observed in roots from the inoculation experiments, in roots from corn plants growing under aseptic conditions in large test tubes and also on fresh clean cane roots placed in sterile water in petri dishes and inoculated with a pure culture. In a few cases in the roots, the oospores have been quite abundant. The mycelium of this Pythium also is quite characteristic. Beside the ordinary type of mycelium, the fungus produces numerous globular, lobed or irregular bodies which may be considerably branched. (Plate XI, E and F.) As these large bodies develop, often the protoplasm from the hypha on which they form flows into them and the hypha is left as an empty tube. Each one of these masses is then separated from the old colony. These large bodies, which are doubtless the same as the enlarged hyphae often seen in cane root tissues (Plate XI, D), have been described by other investigators, including Carpenter and Branstetter. These bodies resemble the prosporangia described by Edson (11), but all attempts to produce zoospores from them have been unsuccessful. As far as has been observed, they germinate by means of small germ tubes. The identity of this Pythium is at present uncertain. In Hawaii, Carpenter is calling the fungus *Pythium aphanidermatum*. As brought out in a previous paper (10), this determination cannot be correct because *P. aphanidermatum* attacks a number of plants on which the sugar-cane Pythium is nonparasitic. Drechsler (7), in a recent paper, also states that the corn and cane Pythiums are distinct from *P. aphanidermatum*.

A culture of a corn Pythium received from Miss Johann of the United States Department of Agriculture and a culture of a Hawaiian-cane Pythium have also been compared with the Louisiana cultures. While similar in many ways, these cultures have shown some differences. Conidia are produced in pure cultures much more readily than in cultures of the

parasitic Pythium from Louisiana. It is not yet possible to state whether or not the cultures are distinct enough to represent separate species.

LOSS OF VIRULENCE

In the course of the work with Pythiums, it has frequently been observed that all cultures of parasitic Pythiums are not equally parasitic. Certain cultures would practically prevent the development of roots of cane or corn in sterilized soil, while others would develop slower, often permitting the growth of a fairly good root system. This condition might mean the presence of different species or different strains or it might mean that the fungus could lose its virulence when grown under certain conditions.

The inoculation experiments carried on during the period of more than two years have possibly helped to explain this condition. One of the early cultures isolated in the summer of 1926 was extremely virulent at the time of isolation. This has been kept in culture on bean-pod agar for more than two years. As it has been used in inoculation experiments almost constantly, it has been possible to observe any change in virulence. For a period of about a year, no change in the virulence was noted. During the second year, however, the virulence gradually declined. After two years, the culture was only moderately parasitic. At that time, even under conditions very favorable for the fungus, corn or cane roots would make a fair growth in inoculated soil. The roots would become somewhat discolored and a few would show the soft flabby tips but the effect was far less marked than with the same culture on an earlier date. Freshly isolated cultures used at the same time, as a check, were as parasitic as this culture was known to be at first. As this culture originally came from a single mycelial thread transferred from a plate, this condition cannot be explained in any other way than that the culture gradually lost its virulence. It is possible that the presence of weakly parasitic forms in the field might be explained in the same way.

DISCUSSION

Although it has been demonstrated that certain species of Pythium are able to cause a severe rotting of cane roots under controlled conditions in sterilized soil, it is not easy to determine the exact relation of these fungi to the root rot as it occurs in the field. In cane fields severely injured by root rot, large numbers of roots may be found showing pale, greyish or brownish, flabby ends. These roots contain mycelium and spores of Pythium and are very similar to roots previously inoculated with pure cultures in sterilized soil.

On the other hand, the surveys made in Louisiana show that these same species of Pythium are widely distributed over the sugar-cane belt occurring

as abundantly, apparently, in fields of thrifty and seemingly healthy cane, as in fields of chlorotic, stunted cane.

Furthermore, it is known that certain varieties of cane are much more tolerant to root rot than others and will make a fair growth in fields in which susceptible varieties will be very badly affected. In Louisiana, varieties like P.O.J. 36 are tolerant, while varieties like Louisiana Purple and D 74 are susceptible. In Hawaii, a similar condition exists and the variety H 109 has practically replaced the Lahaina cane in districts where the Lahaina disease is severe. Yet, in Louisiana, it has been found that roots of varieties like P.O.J. 36 and P.O.J. 213 are readily attacked by species of Pythium in inoculated sterilized soil.

The distribution of root rot in differently located and treated fields and its occurrence from year to year are also of considerable importance. It may be severe in one field and of minor importance in another nearby, and its occurrence in a field one year does not necessarily mean that it will be troublesome with the next crop. Usually, in infected fields, there are areas of good and poor cane. The disease is also very much more trouble some years than others. In some seasons, a very large percentage of the crop shows the root-rot symptoms, while in other years the disease shows only in favored spots.

If species of Pythium are to be recognized as organisms responsible for a considerable portion of the decay of the cane roots, it is necessary to explain why they are sometimes active and other times not. It must necessarily follow that there are other factors present which may make the plant itself more resistant or else make the fungi less effective. Species of Pythium are to a degree facultative parasites and it is reasonable to suppose that their activity as parasites depends to a considerable extent on temperature and moisture conditions, on physical and chemical conditions in the soil, on the reactions of various soil organisms, and on the vitality of the cane itself.

Certain of the factors which influence the development of root rot are fairly well known. For instance, it is known that the disease is always severe following winters of heavy rainfall (9) and that it is favored by poor drainage conditions.

At present, an intensive study is being made of the factors influencing root rot and the parasitism of species of Pythium. These include among others, temperature and moisture conditions and the effect of the application of both organic and inorganic substances to the soil. From these studies it is expected that a better knowledge of the conditions favorable or unfavorable to root-rot development will be determined.

The evidence at present, mainly accumulated from field observations extending over a period of several years and supplemented by results ob-

tained from numerous inoculation experiments, seems to indicate that certain species of Pythium do cause a serious rotting of cane roots and, to a certain extent, cause a killing of the buds, while the cane stalks are covered with soil during the winter and early spring. However, the severity of the attack seems to depend very largely upon the vigor of the cane plants and upon certain environmental conditions such as temperature, soil moisture, and the physical, chemical, and biological conditions of the soil.

SUMMARY

1. A disease of sugar cane known as root rot or root disease occurs in many of the sugar-producing countries of the world. The roots of the cane decay and cause the plants to make a poor and unsatisfactory growth.

2. The ends of the roots and the young laterals are the most subject to decay. Usually the tips of the roots become soft and flabby due to the decay of the stele as well as the cortex.

3. In Louisiana, species of Pythium are usually present in the decaying cane roots.

4. A decay of cane roots in sterilized soil, similar to that which occurs in the field, has been produced consistently in numerous inoculation experiments with pure cultures of Pythium. In the inoculation tests, roots of resistant varieties rotted as readily as did those of susceptible varieties.

5. Certain other members of the grass family, including corn, sorghum, wheat, and oats are attacked by Pythium as readily as is sugar cane. Legumes are not attacked by the same species.

6. Pythium species are readily isolated from cane roots. From the results of thousands of root cultures, it has been determined that these fungi occur in great abundance, being the most common organisms present.

7. All cultures of Pythium obtained from cane roots are not parasitic to cane. In tests about 80 to 85 per cent. have been found parasitic.

8. Parasitic Pythiums occur on roots of both thrifty and stunted cane.

9. Several types of Pythium have been found on cane roots. One of these seems to be of the most importance. Its identity has not been determined.

10. The ability of Pythium to attack cane roots seems to depend to a considerable extent on environmental and other factors.

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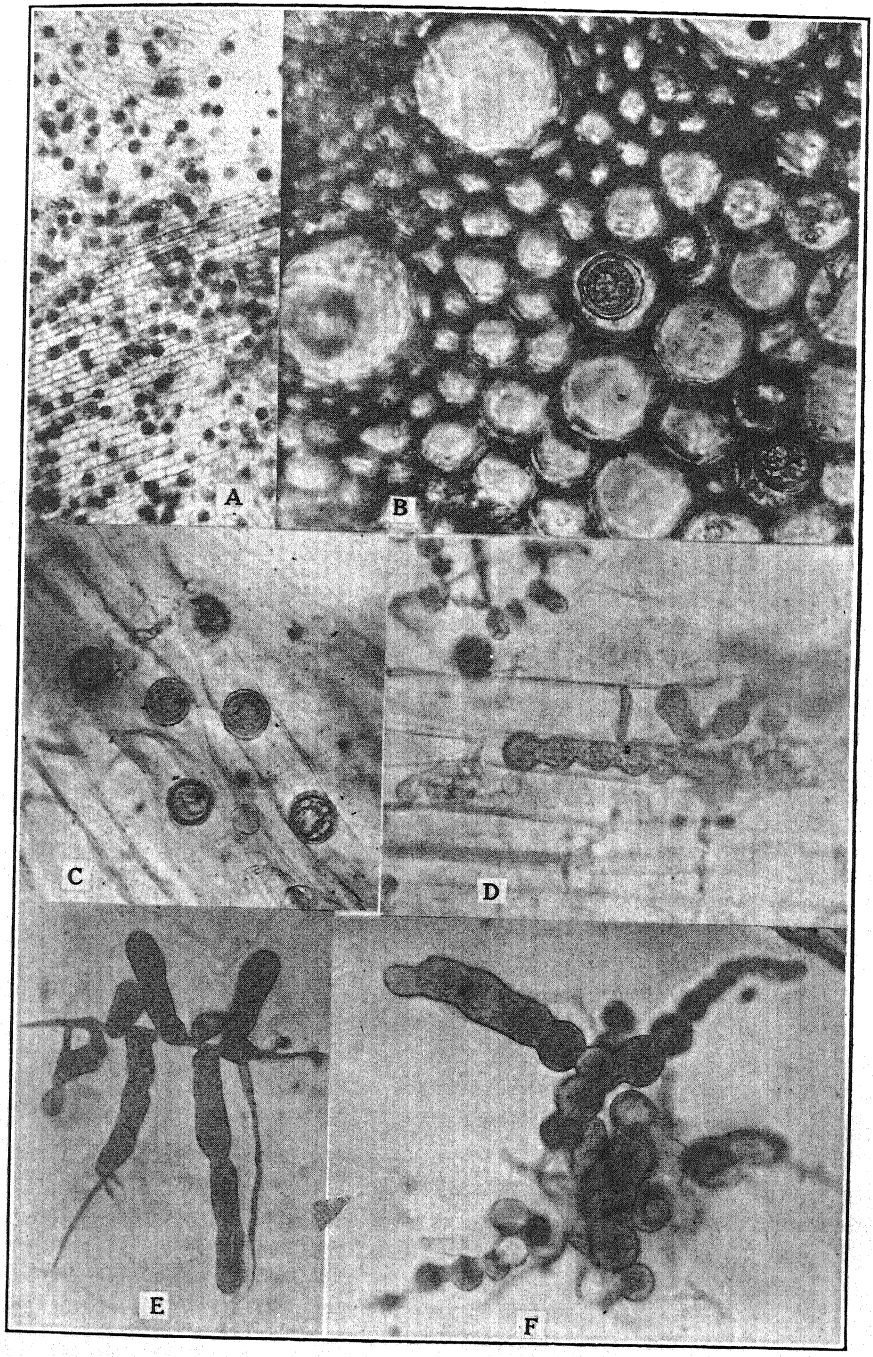
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PLATE XI.

Mycelial and fruiting characters of species of Pythium from sugar cane.

A. Numerous oospores in cane roots. 64X.
B. Oospores in cane roots, natural infection. 353X.
C. Oospores in cane roots from inoculation test. 300X.
D. Swollen cells of Pythium in cane roots from inoculation test. 300X.
E. Swollen cells from Pythium culture isolated in Louisiana. 300X.
F. Swollen cells from Pythium culture from Hawaii. 300X.


A
B
C
D
E
F

PLOT ARRANGEMENT TO DISTRIBUTE NEIGHBOR INFLUENCE IN FIELD TRIALS

CARL HARTLEY

INTRODUCTION

Competition between adjacent plots has frequently been emphasized by agronomists as a disturbing factor in field trials. An excellent brief statement of the difficulties involved is that of Stadler (13, p. 6–8). Competition is by no means the only kind of neighbor influence that has been recognized. Particularly, in pathological and entomological experiments, the results on a plot may be materially influenced by the parasite harbored in a neighboring plot or by the experimental treatment that has been given a neighbor. An especially good example of such interference is found in a series of experiments on rahar (*Cajanus indicus*) described by McRea (8, p. 45, 46). It was found that the wilt caused by *Fusarium udum* spread through the soil 9 feet in a single season. Certain plots were so affected by the wilt's spreading into them from their neighbors that the experiment threw no light on the fertilizer problems for which it had been conducted. In the same way, when parasites of aerial plant parts are present, inclusion of a highly susceptible variety in a variety trial may subject the varieties planted adjacent to it to abnormally heavy infection. This effect may, of course, be offset in part by decreased competition from the diseased variety. A fertilizer, an irrigation, or a soil-disinfectant treatment may have a direct effect on the margins of neighboring plots; the drift of fungicides or insecticides through the air in spraying and dusting experiments may exert a still more distant neighbor influence. R. D. Rands[1] pointed out that some treatments may act as repellants to harmful insects, snails, or other animals and in this way result in intensified infestation of neighboring plots.

By the usual methods of plot arrangement in experiments containing several replications the varieties or treatments are arrayed in approximately the same order in each series. This means that variety C has varieties B and D for neighbors in several different series or perhaps in every series in the experiment. If variety D is especially inclined to influence its neighbors unfavorably, the yield of variety C will be subject to a systematic error that can not be measured or corrected satisfactorily.

A common method of avoiding or reducing neighbor effects has been to leave planted or unplanted buffer strips between the trial plots, or to discard plot borders in taking results. There are a number of objections to such procedure. It places the plots to be compared farther from one an-

[1] Oral communication.

other than they otherwise would be, with consequent decrease in the value of comparisons. If the buffer strips are left unseeded, they sometimes (3, 4) cause certain of the varieties or treatments under comparison to take a higher or lower rank than that to which they are entitled. The use of buffer strips increases the amount of land and labor required for an experiment. In the Beaven arrangement (1), discarded buffer strips occupy 7/16 of the entire field, while in 3-row plots (13), 2/3 of the field is so sacrificed. Furthermore, particularly in the case of pathological factors, the distance through which influence is exerted may be so great or so uncertain that there will be no assurance of eliminating neighbor influence by any practicable width of buffer.

ARRANGEMENT FOR ROWS OR OBLONG PLOTS

In experiments at the Instituut voor Plantenziekten, Buitenzorg, Java, in 1921, the writer was confronted with the need of testing numerous varieties of peanut for resistance to a soil-borne disease, when the only fields known to be infected heavily and uniformly enough for proper comparisons were too small to permit the use of any kind of buffer strips. Single rows were accordingly taken as experimental plots and all attempt to prevent neighbor influence was abandoned. Instead a special planting arrangement was devised to distribute neighbor influence as equally as possible to all the varieties in the test.

To carry out this arrangement completely, the varieties are arrayed in different order in the different replication series in such a way that if the number of replications equals the number of varieties tested, each variety is in contact twice with each other variety. If, then, variety C should prove dangerous to its associates because it carries heavy disease infection or makes unusually heavy demands on soil moisture, all of the other varieties in the test would be equally subject to the misfortune of being in contact with it. All of them would make a poorer showing than if C were not in the experiment, but there would be relatively little chance that the influence of the C plots would cause any one of the other varieties to take a lower rank than it deserved. The arrangement by which this was accomplished is shown in figures 1 and 2. It has been tested in work with sugar cane by Rands and Stevens (11).

One need for refinement was not at first realized; if variety or treatment C exerts an influence on its neighbors, then not only all the other varieties, but also variety C, itself, must be subjected to that influence if comparisons are to be fair. This can be done only by seeing that each variety comes into contact with itself as often as it does with any other variety.

To understand the system followed in this arrangement, the guard plots, indicated in figure 1 by broken lines, should for the present be ignored, and

	Series 1	Series 2	Series 3	Series 4	Series 5	Series 6	Series 7	Series 8
Guard Plot	A	H	G	F	E	D	C	B
Plot 1	A	H	G	F	E	D	C	B
Plot 2	H	G	F	E	D	C	B	A*
Plot 3	B	A*	H	G	F	E	D	C
Plot 4	G	F	E	D	C	B	A*	H
Plot 5	C	B	A*	H	G	F	E	D
Plot 6	F	E	D	C	B	A*	H	G
Plot 7	D	C	B	A*	H	G	F	E
Plot 8	E	D	C	B	A*	H	G	F
Guard Plot	E	D	C	B	A	H	G	F

FIG. 1. Arrangement of an eight-variety test with each variety twice in intraseries contact with every other variety, and with distribution balanced (center of gravity for each variety coinciding with that for the entire experiment). Plots planted to variety A are starred to make this distribution more evident. The plots inclosed in broken lines are guard plots, which are also useful in subjecting each variety to contact with itself; they are discarded in taking the results of the experiment proper.

	Series 1	Series 2	Series 3	Series 4	Series 5
Guard Plot	A	E	D	C	B
Plot 1	A*	E	D	C	B
Plot 2	E	D	C	B	A*
Plot 3	B	A*	E	D	C
Plot 4	D	C	B	A*	E
Plot 5	C	B	A*	E	D
Guard Plot	C	B	A	E	D

FIG. 2. The arrangement shown in fig. 1, adapted to an odd number of varieties or treatments.

attention paid only to the letters in the solid-line plots. In the first series, variety A is put in the first odd-numbered plot, B in the second odd-numbered plot, and so on. The method expressed generally is to go down through the series filling the odd-numbered plots, and then return, filling the even-numbered plots. The second series is started with variety A in the second odd-numbered plot instead of the first. The third series is started with A in the third odd-numbered plot. In series 2, 3, and 4, one gets back to the top of the series with one or more varieties still unused. He disposes of these by coming down again through the still unfilled odd-numbered plots at the top of the series. The last four series in an 8-variety test are a reversal or mirror image of the first 4; the fifth series is begun by putting variety A in the lowest even-numbered plot, going up while filling the even-numbered plots and then down through the odd-numbered ones. In series 6, A is put in the second even-numbered plot from the bottom.

An odd number of varieties is handled in the same general way. This arrangement for a 5-variety test in shown in figure 2. With an odd number of varieties the plan can not be divided into two mirror-image halves, but the same rule is followed that when A has been used in the bottom odd-numbered plot, it is used in the next following series in the bottom even-numbered plot.

Only intraseries contacts are considered here. Field plots are ordinarily much longer than wide; the interseries contacts are between plot ends and usually can be disregarded, or avoided by buffer strips which will be relatively short, as indicated on the diagrams in figures 1 and 2.

The arrangement as shown by the plots in solid lines in figures 1 and 2 fails to provide self contacts. Self contacts have accordingly been supplied by adding guard plots to each series, shown by the dotted lines. It is usually a desirable practice to put at the ends of an experimental series plots that are sown like the experimental plots but whose yields are not considered when the results are taken. By putting in each of these guard plots the same variety as is used in the adjacent test plot, each variety is subjected to as many contacts with itself as it has with any other variety.

Past attempts to avoid error from neighbor influence had in some cases involved the grouping of closely related varieties, and in other cases the use of different orders in different series (13). The only arrangement encountered in which the order was shifted systematically enough to fully equalize contacts within series was the one devised by Richey for another purpose and shown in part by him (12, table 1, columns 2, 9, 10, 11, and 12). As he pointed out, every variety competed an equal number of times with every other variety; an equal number of self-influenced plots for each variety was also provided by his scheme. His arrangement is not entirely balanced, and to carry out his scheme completely the number of replications must be more than twice the number of varieties tested, requiring $(2M^2 + M)$ plots when M is the number of varieties. He did not favor its further use, but suggested the employment of some method that changed the order in different replications.

The arrangement used by the writer is perfectly balanced when the guard plots are ignored. Maskell (9) has emphasized the value of balanced distribution in field trials. For complete balance, the center of gravity for each variety must coincide with the center of gravity of the experiment as a whole. The A's are starred in figures 1 and 2 to make it easier to judge the distribution of replicates. It will be noted that there is one A plot in every rank and every file; as Fisher has pointed out through Maskell (9, p. 380), in any diagrammatically (2) square arrangement this insures the balance of the distribution.

In adapting the proposed arrangement to fields of different shape, the different series may be shifted in position from that shown in figures 1 and 2 without loss of balance so long as their orientation remains the same. If series are placed end to end, proper placing will provide many of the desired self contacts between varieties without need of guard plots. For example, if series 6 of figure 1 is placed below series 2 but without guard plots, the D plots of each series will be in direct contact with each other and both D plots will then be subjected to self-influence.

The proposed arrangement will be more useful in final tests than in preliminary elimination trials, because of the small number of replications possible in the former. If it is only possible to have half as many replications as there are varieties, in such an experiment as shown in figure 1, only series 1–4 would be employed and contacts would still be completely equalized, though balance would no longer be complete. If it were possible to employ but 3 replications, series 1–3 would be followed, and, while contacts would not be entirely equalized, no pair of varieties or treatments would contact more than once, so that there still would be little error due to neighbor influence.

It is sometimes desirable to have more replications of some things than of others. If, for example, 6 different treatments are to be tested and there are to be 2 untreated plots in each series, the experiment is outlined like an 8-treatment test, and untreated plots are put in place of two of the letters, *e.g.*, D and H, shown in figure 1. At first thought such a procedure seems to provide too many self contacts for the untreated plots, but analysis shows that this is not the case.

Because some effects, such as those resulting from shading or from the drift of fungicides with the wind, are exerted mainly in one direction, it is preferable to have even numbers of objectives in an experiment. In figure 1, showing an 8-variety test, it will be noted that of the 2 contacts between any pair of varieties as A and B, A is above in one case and below in the other. In figure 2, showing a 5-variety test, the result is seen that would always occur in an experiment with an odd number of objectives. In both its contacts with B, A is placed below it.

ARRANGEMENTS FOR TREES OR SQUARE PLOTS

In some experiments, square plots are preferred to oblong plots or rows, *e.g.*, centgener experiments and Beaven's chessboard for small grains. In experiments with woody plants or plants grown in hills a single plant or hill may constitute the experimental unit. In all such cases it is desirable to equalize end as well as side contacts; the contacts that appear in the horizontal axis in the chart, as well as those in the vertical axis.

While no arrangements have been developed that would equalize contacts in the ranks of a chessboard and independently equalize them in the

files, it is not so difficult to equalize the total number of contacts when both the contacts within ranks and those within files are added together. The chessboard shown by Orton and Olson (10) for 5-variety tests, while it scarcely lives up to their claim of eliminating experimental error, is excellent for square plots in reducing the portion of the error that results from neighboring influence. In their arrangement variety 2 has varieties 1 and 3 for its only neighbors in the ranks, and varieties 4 and 5 for its only neighbors in the files, so that when one considers both vertical and horizontal contacts each variety in the test competes with each other variety four times. The arrangement is a good one for square plots. For plots that are much longer than wide, contacts in ranks and contacts in files are not of equal importance and their arrangement is not satisfactory.

Orton and Olson make a sweeping recommendation of magic squares in checkerboard arrangements. These are squares such that, if the varieties are represented by numbers, the sums of the figures in each rank, each file, and each of the two main diagonals will be the same. It is by no means

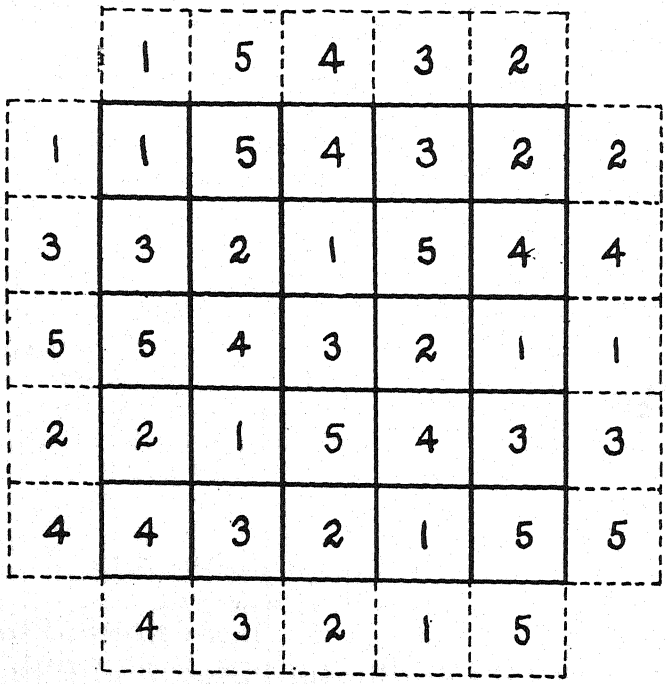


FIG. 3. A five-variety magic square in which each variety comes in contact with each other variety four times, counting both vertical and horizontal contacts. It is taken from Ball (*5*, fig. iv) but has been provided with self contacts by adding guard plots, which are shown by broken lines.

necessary to make a square magic in order to secure good distribution of replicates. Fisher's simpler rule already mentioned (9), that each variety should occur once in each rank and once in each file, is much better. The 6-variety diagram that Orton and Olson show as a magic square, in fact, is not magic because the sums of the diagonals are not equal, but it complies with Fisher's rule and is quite as satisfactory an arrangement as if it were magic. The magic quality gives no assurance of equalization of contacts; nothing could be much worse from the neighbor-influence standpoint than the magic squares obtained by one of De la Hire's methods for first subsidiary squares (5, p. 143), which, if applied to a variety test, would give a variety the same pair of neighbors in both ranks and files throughout an experiment.

A balanced 5-variety square similar to that shown by Orton and Olson is found in Ball's figure iv (5); the arrangement is shown in figure 3, the varieties being designated by numbers instead of letters so that the magic attributes will be apparent. It happens that the writer's 5-variety plan of figure 2 also is magic, while the 8-variety arrangement of figure 1 is not.

Three-variety and 4-variety chessboards with 2-way-contact equalization and balanced distribution are easily devised; samples are shown in figures 4 and 5. Apparently, any 3-variety square that satisfies Fisher's rule (previously mentioned) for balancing the distribution also equalizes neighbor contacts. This is not true for 4-variety squares. The arrangement shown as figure 5 is identical with one shown by Maskell (9, diagram 3), which he dismissed in favor of random arrangements without considering its value in connection with neighbor influence.

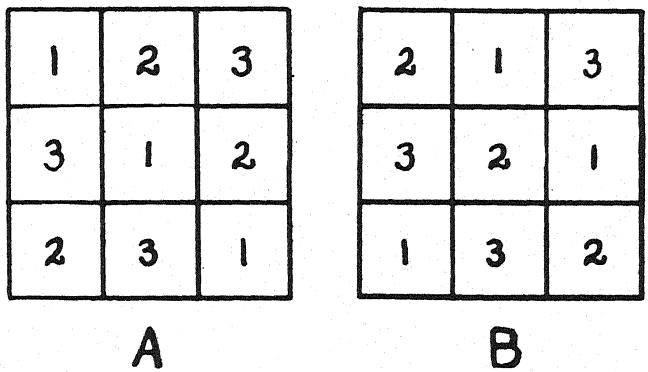


FIG. 4. Three-variety squares, with neighbor contacts equalized when both vertical and horizontal contacts are counted. B is magic, while A is not.

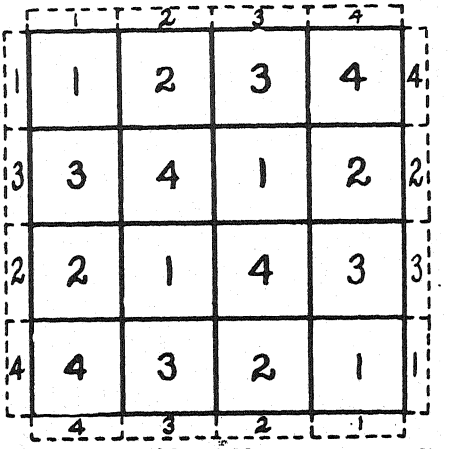


FIG. 5. A four-variety square, with neighbor contacts equalized when both vertical and horizontal contacts are counted. Self contacts are furnished by the guard plots included in the broken lines. The guard plots are made narrow to economize field space.

CONTACT-EQUALIZING VERSUS RANDOMIZED ARRANGEMENTS

Fisher holds randomized plot arrangement superior to any rigid arrangement, because it makes possible a more accurate estimate of the nonsystematic errors. He implies (7, p. 506) that refinement of the estimate of error is more important than the reduction of error.

A contact-equalizing arrangement in experiments in which it makes possible the omission of buffer strips reduces the nonsystematic error both by putting the compared plots closer to each other and by permitting more replications with the same amount of seed and soil. In experiments in which it decreases neighbor influence, it reduces one kind of systematic error, and thereby both decreases the error and increases the accuracy with which the total experimental error can be estimated. Of course the randomized arrangement allows less bias from neighbor influence than would one of the old arrangements in which the varieties of treatments follow each other in the same order in each replication, but would not be so good in this respect as a contact-equalizing arrangement.

The argument of inconvenience raised by Engledow and Yule (6) against randomized arrangement would apply also to a contact-equalizing or any other shifting-order arrangement, but is not believed to be a serious objection. A choice between the two types of arrangement would have to depend somewhat on the amount of neighbor influence expected in the type of experiment to be conducted.

SUMMARY

Competition is by no means the only kind of neighbor influence that may bias results of field trials. This is especially true in experiments on

varietal resistance or prophylactic treatments against diseases or insects.

The use of buffer strips between plots to avoid neighbor influence increases the cost of experimentation; and by decreasing the intimacy of comparisons it reduces their reliability.

Bias due to neighbor effect in replicated trials can be reduced without intraseries buffer strips if each variety or treatment has an equal number of contacts with every other variety or treatment and also with itself. An arrangement is described which attains this end and also satisfies the requirement of balanced distribution.

For small numbers of varieties or treatments it is possible to arrange square plots or single trees or hills in such a way as to equalize or approximately equalize contacts when both interseries and intraseries contacts are counted.

Contact-equalizing arrangement permits a less accurate estimate of nonsystematic error than Fisher's randomizing arrangements; but, under certain circumstances, may so reduce systematic error as to make the computed error more nearly representative of the total experimental error.

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DASYSCYPHA FUSCO-SANGUINEA REHM ON WESTERN WHITE PINE, PINUS MONTICOLA DOUGL.

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During the last eight years, 1921 to 1928, while scouting for white-pine blister rust, *Cronartium ribicola* Fischer, in the white-pine belt of northern Idaho, northeastern Washington, and northwestern Montana, the writer observed, or had called to his attention, a parasitic disease on western white-pine, *Pinus monticola* Dougl., the gross characteristics of which, on the younger trees, appear in several respects very much like white-pine blister rust. The two are so similar in general appearance that on a superficial examination one may be mistaken for the other. In fact, several areas of western white pine infected with the disease have been erroneously reported as infected with blister rust. Because of the gradual invasion of the white-pine belt by white-pine blister rust and the desirability of locating initial infections of it, any disease which might be confused with it should be reported. Further, since the disease under notice has not within the writer's knowledge been reported as an active parasite on western white pine, it seems worth while to record the following observations on its characteristics, importance, and distribution.

This disease is of still further interest due to its similarity to that caused by the destructive larch canker, *Dasyscypha calycina* (Schum.) Fckl., which has recently been found in the eastern part of the United States (5).

CAUSAL ORGANISM

In nearly all cases where this disease has been observed, a Dasyscypha has been found on the cankers. Saccardo (4) reports over one hundred and fifty species in this genus, but only a small number of them are said to be even mildly parasitic. The only vigorous parasite thus far reported is *D. calycina.*

Underwood (7) reports *Dasyscypha calycina* on *Pinus* sp. Tubeuf (6) says *D. calycina* may parasitize pines and that it occurs in America. Wagner (8) reports *D. calyciformis* (Willd.) Rehm as parasitic on *Pinus sylvestris* L., *Abies pectinata* DC., and *Picea excelsa* Link. Anderson (1) reports *D. resinaria* (Cooke and Phillips) Rehm as a parasite causing cankerous growths on *Abies balsamea* Mill. in Minnesota. According to Jelstrup (3), Jorstad has noted deep cankers on *P. sylvestris, P. montana* Mill. and *P. murrayana* Balf., caused by *D. subtilissima* Sacc. He also found *D. resinaria* on *P. sylvestris.* Of all those which have been reported as

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parasitic, the larch canker, caused by *D. calycina,* is the only one which has been recorded as a vigorous, destructive parasite. A very complete discussion of this parasite is given by Hiley (2).

The Dasyscypha under discussion in this paper is such a vigorous parasite in some areas that it was first thought to be the larch canker established on western white pine in this region. For this reason in all areas where this disease has been observed on white pine, a search has been made for it on larch as well as on other trees. In no case was this fungus definitely found on other species of trees in an area where it occurred on white pine. In northwestern Montana during the summer of 1928, however, a Dasyscypha was found parasitic on a single tree of western larch, *Larix occidentalis* Nutt. A small trunk canker had developed on a young tree about two inches in diameter. No other infections on larch were found. This species appeared to be the same as that on white pine. The measurements of the spores were within the range of those of the white-pine organism, but the color of the apothecia was much lighter.

On a fresh specimen of the white-pine Dasyscypha the stipe and under side of the cup are light brown, while the inside of the cup is a deep orange. On dry specimens these colors become darker. The length of the stipe as well as the diameter of the cup vary greatly. The edges of the cup inroll when the specimen dries or has matured, completely closing the cup so that the interior is not visible. The spores are hyaline, generally ovate-elongate, uniformly 6 μ in diameter but varying considerably in length, mostly within the limits of 16–20 μ. The asci are clavate and hyaline, the majority measuring within the limits of 115–120 × 8–10 μ. The paraphyses are generally 10–20 μ longer than the asci and are 2 μ in diameter. The hairs are yellowish brown. All measurements were made from sections of mature apothecia mounted in a solution of one-half per cent sodium hydroxide.

This fungus approaches the measurements given for *D. calycina* and *D. fusco-sanguinea,* but differs from each in certain particulars. Its ascus and spore measurements agree with the original description of *D. calycina* but do not agree in respect to color of hairs and inrolled margin. On the other hand, the measurements for *D. calycina* as given by Hiley are much larger than those given in the original description for *D. calycina.* Since Hiley made an extensive investigation of this species, his measurements probably represent more nearly the true measurements of *D. calycina* in its parasitic form. If this view be accepted, the asci and spores of the white-pine fungus are too small to be those of the parasitic form of this species. Evidently the original description was based upon the saprophytic form of *Dasyscypha calycina* since specimens of this fungus on very small twigs of the larch which were collected in England in 1927 by Dr. Boyce of

the Office of Forest Pathology agree with the original description. In the color of the hairs and the inrolled character of the apothecia, this parasite agrees with *D. fusco-sanguinea,* but the sizes of the spores and asci are considerably larger than those given in the original description for this species. The following table gives a comparison of the asci, spores, paraphyses, and hairs of the four forms:

COMPARISON OF PARASITIC AND SAPROPHYTIC FORMS

Species	Asci (μ)	Spores (μ)	Paraphyses	Hairs
1. D. calycina (Schum.) Fckl. (probably saprophytic)	120 × 9	16–22 × 6–7	Hyaline, 2.5 μ	Hyaline
2. D. calycina fide Hiley (parasitic)	140–200 × 9–15	17–23 × 8–10	Hyaline, 2 μ	Hyaline
3. D. fusco-sanguinea Rehm. (probably saprophytic)	70 × 10	15 × 5	Hyaline, 2 μ	Reddish brown
4. D. sp. white-pine species (parasitic)	115–120 × 8–10	16–20 × 6	130–140 × 2 μ	Reddish brown

It is a fair inference from the study of this table and the facts previously stated that Number 4 bears to Number 3 the same relation that Number 2 bears to Number 1 and, notwithstanding the variations, the fungus on our western white pine may very properly be accepted as the parasitic form of *D. fusco-sanguinea.* This opinion is concurred in after independent investigations by Weir, Diehl and Dearness.[2]

DISTRIBUTION

The writer first had this disease called to his attention as white-pine blister rust in August, 1921, by a forest ranger on Fish Creek in Montana, at the western border of Glacier National Park. Since that time, its distribution has been found to be general in the white-pine region. The more important locations where it has been observed are given in this paper. As a rule, the most heavily infected areas occur at relatively high elevations, that is, 4500 to 6000 feet, on sites where white pine is near its altitudinal limits or otherwise on a site rather unfavorable for white-pine growth. However, it has been observed in young reproduction in some localities on fairly favorable white-pine sites, attacking about 20 per cent of the trees. At lower elevations in other parts of the region, it occurs only as an oc-

[2] The writer wishes to express his thanks to Dr. J. R. Weir and Mr. W. Diehl of the Office of Mycology and Disease Survey, United States Department of Agriculture, and to Professor John Dearness, Ontario, Canada, for examining specimens which were submitted to them.

casional infection on the smaller limbs, killing these, but causing no other evident damage.

The following are records of collections and observations of *Dasyscypha fusco-sanguinea* on white pine, *P. monticola.*

Montana:

Fish Creek, west edge of Glacier National Park, August, 1921. On trees about 10 feet tall. Fifty per cent of the trees infected. A few trees had dead tops and some were dead. Specimens on file at Forestry School, University of Idaho, Moscow.

Idaho:

Gold Creek, Section 17, Township 40 N., Range 3 E., Boise Meridian, Aug. 5, 1923. Large trunk cankers on trees 4 to 5 inches in diameter. Trees were evidently overcoming the disease.

Stoney Creek, Section 17, Township 41 N., Range 3 E., Boise Meridian, Aug. 10, 1923. Trees 1 to 7 inches in diameter, on about 2 acres, 75 per cent infected and several dead or dying. No. 2554.[3] (Fig. 1.)

Along trail Freezeout to Leu Boehls, Township 41 N., Range 2, 3, and 4 E., and Township 42 N., Range 3 and 4 E., Boise Meridian. Observed occasionally on branches of trees 4 to 8 inches in diameter. Aug. 11–12, 1923.

Upper Priest River, Section 24, Township 65 N., Range 5 W., Boise Meridian. July 30, 1924. Large basal, trunk cankers on trees 18 to 24 inches in diameter. Ascocarps found occasionally on the cankers. Elevation, 4000 feet; Section 18, Township 65 N., Range 4 W., Boise Meridian, July 30, 1924. Several acres in infected area. Trees 1 to 6 inches in diameter, about 75 per cent infected. Some tops and trees dead. Elevation, 4000–4500 feet. No. 2220 and 2594; Section 29, Township 65 N., Range 5 W., Boise Meridian, August, 1924. Occasional infections on young trees. Elevation, 4000 feet. Report and collection, P. S. Simcoe. No. 2595.

Shedroof Lookout, Section 36, Township 40 N., Range 45 E., Willamette Meridian, August 12, 1924. About 90 per cent of the pines under 15 feet high, infected. Seventy-five per cent killed. Several acres. Elevation, 4500 feet. Observed and reported by H. N. Putnam. No. 2598.

Upper Lionhead Creek, Sections 1 and 12, Township 62 N., Range 3 W., Boise Meridian, August, 1925. Trees, seedlings to poles. Nearly all infected. Observation by H. L. Whiting.

Green Mountain, Sections 1, 2, 11, 12, Township 40 N., Range 3 E., Boise Meridian. Trees 16 to 20 inches in diameter with large basal cankers. A belt about 100 yards wide on the south and southeast slope. Elevation, about 4500 feet. (Pl. XII, B.)

Bear Skull, St. Joe National Forest. September, 1927. Abundant all along the ridge. Trees 50 per cent infected or dead. Has general occurrence at 5500–6000 feet elevation in this region. Observation and collections by P. Rowe. No. 2600.

Yellow Dog Creek, Section 20, Township 23 N., Range 3 E., Boise Meridian, September, 1925. Spotted infection on trees 6 to 8 inches in diameter. Elevation, 3700 feet. Observed by J. R. Rodner.

[3] Numbers are for specimens in writer's herbarium.

Clarkia, Section 4, Township 42 N., Range 2 E., Boise Meridian, September, 1925. Infection occasional on branches of trees 6 to 8 inches in diameter. Elevation, 3200 feet. Observation by J. R. Rodner.

Quartz Creek, Sections 25 and 26, Township 58 N., Range 5 W., Boise Meridian, November 16, 1927. Occasional on trees 5 to 10 feet tall. Observed by C. C. Strong and P. S. Simcoe.

Washington:

North Baldy Mountain, Section 8, Township 36 N., Range 45 E., Williamette Meridian, July, 1925. About 50 acres on eastern exposure. Trees, seedlings to 4 inches in diameter. Nearly every tree infected and 50 to 75 per cent dead. Elevation, 4500 feet. Reported and collected by H. L. Whiting. No. 2597.

Mount Spokane, Northeast of Spokane, May 30, 1926. Fairly common and well distributed but not severe enough to cause much damage. Elevation, 5500 feet. Observation and collection by H. N. Putnam. No. 2599.

British Columbia:

Nelson, July 10, 1927. On many white pines 3–8 feet high. Many dead twigs. Observation and collection by J. W. Eastham. No. 2601.

Occasional limb infections have been observed in several other localities. It is needless, however, to record other smaller, infected areas which have been observed, since it is apparent from the above records that the parasitic form of *D. fusco-sanguinea* is quite generally distributed over the white-pine area of this region.

The writer has studied and collected specimens of Dasyscypha on conifers in the Northwest wherever they have been observed in the field, thinking that possibly *D. fusco-sanguinea* might occur on other trees or might be an ordinary saprophyte of this group which had found an especially congenial host in the white pine and thus had become an active parasite. In this connection the following collections have been made and are reported for the first time:

Dasyscypha agassizii (B. & C.) Sacc.; on *Pinus monticola* at Daisy Lake, B. C., June 21, 1924. No. 2064,[4] on *Pinus monticola* in Upper Priest River Valley, Idaho. July 31, 1924. No. 2221. Saprophytic and fruiting on dead branches.

Dasyscypha arida Phillips; on *Pinus contorta* Dougl., Warren, Idaho. August 24, 1920. No. 688; on *Abies lasiocarpa* Hook., Warren, Idaho. August 25, 1920. No. 698; on *Abies lasiocarpa*, Lookout Mt., Idaho. August 27, 1925. No. 2474; on *Picea engelmannii* Engelm., Upper Priest River Valley, Idaho. August 22, 1925. No. 2450; on *Pinus albicaulis* Engelm., Lookout Mt., Idaho. August 27, 1925. No. 2485. A common saprophyte, at the higher elevations, on dead branches and trunks of dead trees which have fallen into moist places, the species has a rather misleading name, since in this region it has been observed in such damp situations.

Dasyscypha calyciformis; on *Larix occidentalis*, Priest River, Idaho. May 12, 1920. No. 1412; on *Abies grandis* Lindl., Bungalow, Idaho. June 22, 1923. No.

[4] Numbers refer to collection or specimen number in writer's herbarium. Determinations for most of the collections have been checked by John Dearness, London, Ontario, Canada.

2297; on *Pinus monticola* Dougl., Cheekye, B. C., May 9, 1927. No. 2602. This species is ordinarily a common saprophyte, occurring on dead branches that still remain on the tree. In some cases, it appears to be a very weak parasite but, generally, there is some evidence that the attacked branch is in a weakened condition due to dense shade or injury of some kind. Occasional on blister-rust cankers.

DESCRIPTION OF THE DISEASE

The method of attack appears to be through the needles or needle fascicles, since the early initial infection areas are found as shrunken circular spots around the fascicle of the needle (Pl. XII, C). The bark shows distinct depressions for each year's growth (Pl. XII, A) due to the shrinking and drying out of the bark, so that the rate of spread of the disease each year may easily be determined. The rate of growth of these areas gives an indication of the resistance of the tree to the attack of the fungus. Very often there is a distinct cracking of the bark at the end of the season's growth. This is especially true if the fungus experiences difficulty in making progressive growth in the tree (Pl. XII, E). Occasionally this cracking at the point of farthest advance of the fungus may show signs of the formation of callus. The distinct separation by cracking of the growth of different years and the formation of callus becomes more evident as the diameter of the tree increases. On very young twigs the fungus will soon kill the branch, and the bark shows the general cracked condition (Pl. XII, D). The fungus appears to develop about twice as fast longitudinally as it does laterally. It apparently is not able to girdle trees over four inches in diameter. Field observations indicate that the larger the limb or trunk attacked, the less probable it is that the fungus will be able to girdle it.

The typical orange yellow apothecia appear on the area attacked the previous year; in other words, the fungus fruits on the bark in which its mycelium is in the second year of its growth. In the case of the smaller branches, girdled and killed the first year, no apothecia may appear, or, they may appear only on the first part of the canker, indicating that the fungus is primarily a parasite. Fruiting may occur on the several years of growth of the canker and appears to continue on a particular year's growth until the bark dries up and cracks, indicating that the tissue in the area is dead. This dead bark adheres quite firmly to the wood underneath. Due to cutting off of food supply from above there is generally a swelling of the tree above the point of attack. This swelling is especially prominent at the nodes when the main trunk is attacked through one of the nodal branches or just below this point. There is generally an abundant exudation of pitch from cankers. In many cases, especially those of trees over four inches in diameter which have trunk cankers, the fungus seems unable to kill the tissue in the infected area, but continues to grow there year after year

(Pl. XII, A). Evidently the fungus grows in the meristematic tissue, causing a swelling of each year's woody tissue, thus causing a long, oblong swelling in the wood itself. An examination of the wood in these oblong swellings reveals distinct resinous, blackish brown layers in the spring wood, typical for such parasites. In such cases it appears that the fungous growth evidently causes the swelling of both the woody and bark tissue.

There appears to be a limit to the size of the trees killed by this disease or on which the disease can make definite lateral and longitudinal progress. On large trees a deep-seated canker is formed similar to those described for the larch canker. Many trees 6 to 8 inches in diameter have been observed with these deep-seated cankers. Apparently in these cases the fungus in the canker had been unable to spread laterally or longitudinally to any extent, but continued to grow in the meristematic tissue that developed in each new year's growth, killed it, and thus prevented the tree from healing over the wound. Several trees 4 to 8 inches in diameter have been observed, so reduced in mechanical strength at the point of the canker that some had bent over at a right angle toward the cankered side. Figure 1 shows the enlargement on such trees opposite the canker and the partial consequent weakening of the tree.

Trees 10 to 30 inches in diameter have been found with deep-seated cankers about their bases but they evidently had been able to overcome the attack and continue growth, so that the fungus had done little more than distort the tree at the canker. Plate XII, B, shows a typical instance. In these cases the fungus appears to continue to grow and to kill the meristematic tissue at the edge of the canker, thus preventing the tree from healing it over. This causes a shrunken, distorted area. The cankers occur on no particular side of the tree nor at a regular height among any group of trees. No cankers, however, have been observed more than eight feet from the ground in the case of large trees. Large scales of dead bark are found near the edges of these cankers, showing that the disease evidently counteracted the efforts of the tree to heal over the wounds. When the bark is removed, a canker several inches deep is revealed. It is generally elliptical, a few inches in diameter at the bottom of the canker, and several times as large in diameter at the outer edge. The depth of the canker, of course, depends on the diameter of the tree. The canker resembles a minute amphitheater. In all cases examined, it has been observed to extend to within four or five annual rings of the center of the tree, indicating early infection of the tree.

Two areas where practically all of the white pines were thus cankered have been observed on trees 10 to 30 inches in diameter; one on Green Mountain, about 12 miles east of Elk River, Idaho, the other on the west slope of the Upper Priest River Valley, Idaho, in the Kaniksu National

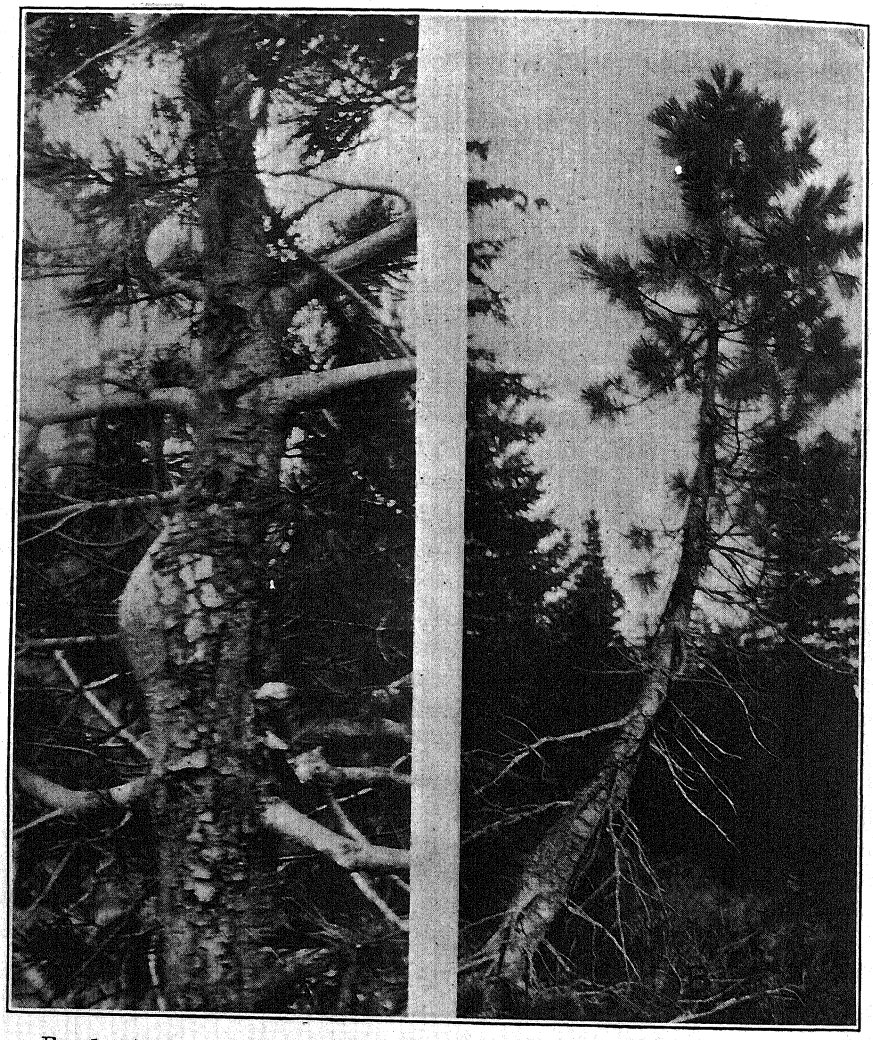


FIG. 1. A, shows a white-pine tree distorted by the Dasyscypha canker. The enlarged portion is directly opposite the canker; B, the enlarged bole at base of tree and limbs being killed by Dasyscypha. No canker occurred on this tree but abundant apothecia developed on the bole and limbs.

Forest. These areas are at elevations of about 4500 feet and hence are near the upper limits of the white pine. Ascocarps of the fungus are not commonly found on these large cankers. On the Upper Priest River area, however, ascocarps were found occasionally. Since these cankers are so similar to those described for the larch and, since similar fruiting bodies have been found on them, it is reasonable to believe that they are caused by the fungus.

Indeed, they coincide quite closely with those described by Hiley (2) on larch. The infected woody tissue, whether the tree be large or small, is impregnated with pitch, making it difficult or impossible to remove sections with a hand axe. In the trees attacked, the butt log, or at least the first eight feet, will be lost entirely.

COMPARISON WITH WHITE-PINE BLISTER RUST

The white-pine-blister-rust canker produced on older trees is very distinct from the canker produced by the parasite, *Dasyscypha fusco-sanguinea*. The old blister-rust canker is characterized by broken, curled, cracked bark on the surface of the tree trunk. The Dasyscypha canker is a deep-seated, distorted cavity, extending nearly to the center of the tree, the cavity appearing as a minute amphitheater. In both cases there is a considerable development of resin in the wood and exudations of it from the cankers stream down the trunk. The typical aecia of blister-rust will generally develop in the bark which has not yet been killed, and the extension of the mycelium into the living bark produces a distinct brownish discoloration. The apothecia of the Dasyscypha on the cankers on older trees are very infrequent and occur on the very edge or slightly inside the edge of the canker.

There is much more similarity in the gross characters of the two cankers as they occur on the younger trees. The incipient stages of the Dasyscypha infection produce a brownish, shrunken condition of the bark. Blister rust causes an orange discoloration of the bark, generally accompanied by a slight swelling of the whole area. This is the stage when the greatest care must be exercised if the two are not to be confused.

After one year's growth, the two may be easily distinguished. Each year's growth of the Dasyscypha is definitely indicated by a marginal ring either of broken bark or by a slight raised area at the edge of the year's growth, due to the fact that the bark at the edge did not shrink as much as toward the center of the canker. Further, the apothecia appear abundantly on the past year's growth. On the blister-rust canker the yellowish brown pycnial spots will be found on the area where the fungus has been present for more than one year and, soon after, the bark is broken by the extruding aecia.

There may be an abundant flow of resin in both cases when young trees are attacked to such an extent that the differences mentioned above are somewhat obscured. By a careful examination of the infected area, however, by one who knows the two diseases, no real difficulty will be encountered in determining which organism is present.

SUMMARY

A common canker of western white pine, *Pinus monticola,* which is being confused with cankers of white-pine blister-rust, is here reported for the first time.

The canker is generally distributed over the white-pine region of the Inland Empire.

The cause of the canker is believed to be a parasitic form of *Dasyscypha fusco-sanguinea.*

The canker is very similar to the larch canker caused by *D. calycina.*

It has many gross characteristics similar to those of the white-pine blister rust.

It is a disease of secondary importance causing considerable damage in white-pine sites favorable to its development.

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PLATE XII

A. Apothecia, rings of annual growth of the fungus, and swollen node.

B. A white-pine tree with two Dasyscypha cankers on the trunk. Typical of infection on Green Mountain east of Elk River, Idaho.

C. Typical infection spots at needle fascicles on a small branch.

D. Young branch dying from Dasyscypha attack. Brownish needles removed in order to show cankered bark.

E. Formation of callus at edge of annual growth. The extent and growth of the fungus, however, are not strictly confined to the swollen portion of the branch.


A
B
C
D
E

RHIZOCTONOSIS OF LOBELIA[1]

S. C. TENG

In the summer of 1927, Professor H. H. Whetzel called the writer's attention to a serious trouble of the double blue Lobelia (*L. erinus* L.) in the greenhouse of the Floriculture Department at Cornell University and suggested that an investigation of the disease be undertaken. It is hoped that the observations and experiments here reported may serve to call attention to a disease probably responsible for much of the difficulty commonly experienced in growing the double Lobelia in the greenhouses. What is probably the same disease has been reported by Rant[2] on *L. erinus* growing in his garden in Java.

SYMPTOMS AND ETIOLOGY

The characteristic symptoms of the disease under humid conditions in the greenhouse are the hydrolysis and yellowing of the lower leaves and the formation of necrotic lesions at the base of the stem. There follows a rotting of the affected organs and the consequent wilting and death of the parts above (Pl. XIII, A). While rotting, the plants often show a thread-blight aspect, the mycelium of the fungus growing all over the plant. Sometimes abundant aerial roots are formed just above the lesions at the base of the stems. After the death of the tops, new shoots may develop from the crowns. In some cases the plants become stunted and exhibit an abnormally dark green foliage.

A Rhizoctonia was isolated from the diseased Lobelia plants. Its characters agree in general with those described for *Rhizoctonia solani* Kühn. In comparison with a strain of *R. solani,* which causes the wire stem of cabbage, and another isolated from a potato tuber, the Lobelia strain has a somewhat higher optimum temperature for growth, about 27° C. The test was carried out in triplicate on both Czapek's agar and potato-dextrose agar. The effect of temperature on the growth of these strains was measured by the increase in diameter of thalli in 24 hours. The optimum growth temperature for the other two strains is about 24° to 25° C. When grown on the same culture medium and at temperatures optimum for each, the Lobelia fungus grew about twice as rapidly as the organism from potato. It also outgrew the cabbage Rhizoctonia by a wide margin, the ratio being approximately 3:2. Two days after planting on potato-dextrose agar in petri dishes at 27° C., the mycelium was found to cover the whole plate; and five days after planting, the formation of sclerotia became evident. The sclerotia were larger

[1] The writer acknowledges his indebtedness to Professors H. H. Whetzel and H. E. Thomas for their valuable suggestions and encouragement in connection with this investigation.

[2] Duggar, B. M. Rhizoctonia Solani in relation to the "Mopopilz" and the "Vermehrungspilz." Ann. Missouri Bot. Gard. 3: 1–10 (See p. 8). 1916.

in size and fewer in number than those of the other two strains (Pl. XIII, B, b).

The fungus makes more rapid growth and produces more sclerotia on Czapek's agar than on potato-dextrose and oat agars, (Pl. XIII, C). The difference between the growths on the two latter agars is not striking. The mycelium in culture is rather appressed. That on Czapek's agar is of a more or less fawn color; while on potato-dextrose and oat agars, it is pale ochraceous buff and light buff (Ridgway), respectively. An important characteristic of the fungus is that it forms one large sclerotium, often 1 to 1.5 cm. across, at the center of the plate and smaller sclerotia in a zone surrounding the center and again at the margin of the plate. The sclerotia are usually rather wooly and vary in color from wood brown to cinnamon brown. Immature sclerotia usually exhibit prominent drops of brownish liquid on their surfaces. The description of the cultural characters of the fungus is based on 16-day old cultures incubated at 27° C.

Late in the summer a partially yellowed leaf near the base of one of the diseased plants was found covered to the base of the petiole with a loose mat of ashy gray mycelium. Freehand sections showed that this weft of mycelium consisted of basidia arising from typical Rhizoctonia hyphae. The writer failed to get cultures of the Rhizoctonia from the basidiospores. However, a portion of the material was preserved in alcohol, and slides were prepared from this by the ordinary paraffin method. These clearly showed the fruiting stage of the fungus which agrees closely with the description of *Corticium vagum* B. & C. as given by Burt.[3]

PATHOGENICITY

The pathogenicity of the fungus was readily established by inoculation experiments upon double blue Lobelia. All inoculations in this investigation were made by the use of steamed wheat kernels bearing a heavy growth of the fungus and placed about the base of the plant below the surface of the soil. In every case the soil or sand was steam sterilized. All experiments were carried out in a greenhouse having an average temperature of 70° F. The soil used was a silty loam.

The first experiment was made on full-grown plants in pots. After inoculation, they were covered with bell jars and generously watered. The plants showed evidences of infection in two weeks. The fungus was reisolated from the diseased plants, and about a month after death and the removal of the diseased plant, it was also recovered from the soil in which the plants had grown. The method of isolation from the soil was as follows: The soil was put into a sterile preparation dish and watered with sterile water. Seeds of cucumber, which had previously been found to be suscepti-

[3] Burt, E. A. The Thelephoraceae of North America. XV. Corticium. Ann. Missouri Bot. Gard. 13: 173–354. 1926.

ble to this fungus, were sown in this soil. The seed was superficially sterilized in calcium hypochlorite before sowing. The seedlings promptly became infected and the fungus was isolated from these in the usual manner.

In a further experiment, cuttings from apparently healthy Lobelia plants were planted in sand planted with the fungus. Within a week, 39 of the 40 cuttings inoculated were prostrate, while of the 60 cuttings in the control flat, 58 lived. The two that died in the check flat showed no evidence of Rhizoctonia.

After having established the pathogenicity of the fungus on the double blue Lobelia, its pathogenicity to four other cultivated Lobelias was tested. Full-grown plants as well as seedlings were used. The former were inoculated by placing infested wheat kernels in the soil, as before described. They were then covered with bell jars. Seedlings were inoculated at the time of transplanting into pots. It was found that *L. erinus* var. *gracilis* is resistant to the attack of the fungus. *L. erinus* var. *speciosa* (White Compact) and *L. ramosa* Benth. succumb when the roots are wounded and the plants put under abnormally moist conditions. Some of the cardinal flowers (*L. cardinalis* L.) became distinctly stunted.

The susceptibility of certain vegetables to the Lobelia Rhizoctonia was also tested. Infested wheat kernels were mixed with the soil just before planting the seed. Severe damping-off was produced by the fungus on cucumber seedlings (Early Fortune). On seedlings of red kidney bean considerable damping-off and stem rot were produced. The fungus was reisolated from diseased bean and cucumber plants. Cabbage (Copenhagen Market) and beets (Crosby's Egyptian) showed moderate susceptibility, while pepper (Ruby King) was found to be resistant in these tests. On both beet and cabbage seedlings a wire stem effect was produced.

CONTROL

Laboratory experiments to test the fungicidal effect on the Lobelia strain of Rhizoctonia were made with sulphur (Niagara Kolodust), aluminum sulphate, copper carbonate, mercuric chloride, Uspulun, Bayer Special II–6 (6 per cent nitrophenol mercury), Bayer Special 144 (4 per cent nitrophenol mercury), and Bayer Special 154 (mixture of 6 per cent chlorophenal and 8.2 per cent nitrophenol mercury). These tests were made by dusing petri-dish cultures of the fungus with the above-named chemicals, and taking the growth of the fungus after dusting as a criterion of the effectiveness of the chemicals. All the mercury compounds tried were found effective. There was no increase in the diameter of the thallus after dusting. Copper carbonate was not so effective, aluminum sulphate still less, and sulphur the least.

Dusting the soil in which double blue Lobelia plants were growing in the greenhouse with various mercury compounds was then tried. Where

resulted. This difficulty was overcome by using the chemicals in solution or in suspension in water. Mercuric chloride, being much cheaper than the organic mercury compounds, was selected for further experimentation. Wiant[4] reports that aluminum sulphate is effective in controlling *Rhizoctonia solani* in beds of *Pinus resinosa* Torr. This material being exceedingly cheap was, therefore, also selected for further studies. It may be applied in either dry or liquid form.

Cabbage and cucumber, being readily grown and also susceptible to the attack of the Lobelia Rhizoctonia, were used in an experiment to determine the dosage of mercuric chloride and of aluminum sulphate necessary to check the activity of the fungus. It was found that three-tenths of a gram of mercuric chloride in a liter of water applied at the time of sowing to each 1½ square feet of space is effective. Five-tenths of a gram of mercuric chloride caused slight and higher concentrations distinct chemical injury to the plants. Aluminum sulphate was found ineffective in controlling the fungus. At a concentration of twenty grams to each 1½ square feet of space, it was evidently injurious to the plants. It was found that double Lobelia plants can withstand five-tenths of a gram of mercuric chloride to each 1½ square feet of space without evident injury. Therefore, for heavily infested soil, one tablet of mercuric chloride in a liter of water to an ordinary 12″ x 18″ flat should be a safe dosage. For slightly infested soil or "unclean" sand one tablet in two liters of water, applied at the rate of one liter to a flat, probably will suffice.

The fungus easily gains ingress to its suscept below the surface of the soil, especially at the time when the latter is propagated in the form of cuttings. Unless clean sand and soil are used, the application of mercuric chloride should be made at the time of propagation and of transplanting. The use of cuttings from healthy plants for propagation is to be recommended. Various observations seem to indicate that humid conditions favor the development of the disease. Any cultural method that will reduce this factor to a feasible minimum will also help to lessen the ravages of the disease.

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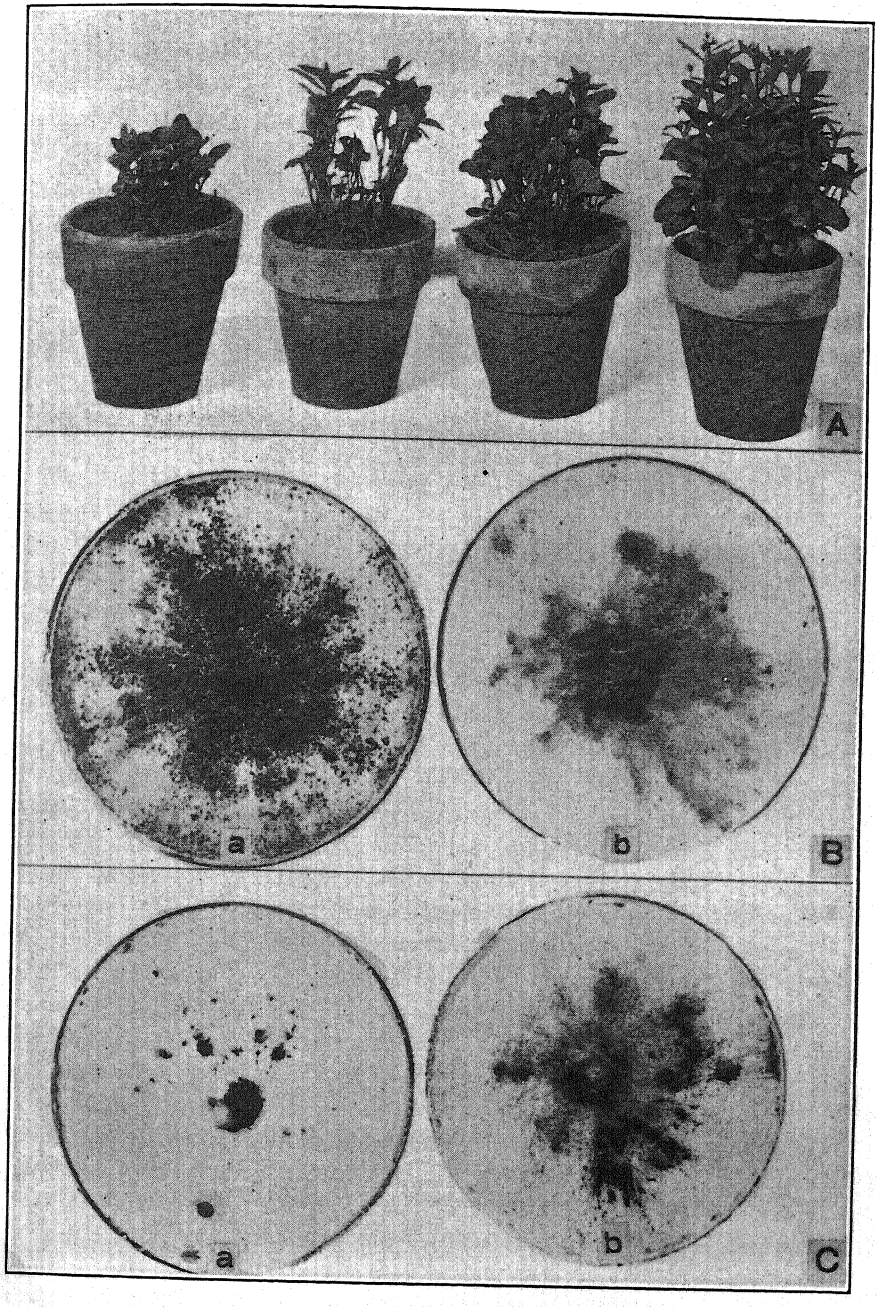
PLATE XIII

A. Showing symptoms of the disease on potted plants of double blue Lobelia (*L. erinus*). The plant on the extreme right is healthy.

B. Showing differences in cultural characters of the potato strain (a) and the Lobelia strain (b) of Rhizoctonia on Czapek's agar. Cultures 16 days old grown at 25° C.

C. Showing cultural characters of the Lobelia strain of Rhizoctonia grown on oat agar (a) and on Czapek's agar (b) at 27° C.; cultures 16 days old. This strain grows more vigorously and produced more sclerotia on Czapek's agar.

[4] Wiant, J. S. Soil treatments for the control of damping-off in coniferous seedbeds. (Abst.). Phytopath. 17: 51–52. 1927.


A
a
b
B
a
b
C

FALSE SMUT OF MAIZE, USTILAGINOIDEA

R. J. HASKELL AND W. W. DIEHL

Early in September, 1925, C. W. Edgerton, of the Louisiana Agricultural Experiment Station, transmitted some corn tassels bearing a fungus that had the appearance of the common smut, *Ustilago zeae* (Beckm.) Ung. A closer examination, however, showed that the various excrescences on the inflorescence were unlike those caused by corn smut, being decidedly different in form, size, color, and texture. An examination of the spores, which completely covered the surface in a smutty mass, showed that the fungus was a species of Ustilaginoidea. This material had been gathered by J. G. Richard, County Agent, at Houma, Louisiana, in August, 1925, and constituted the first report of this disease to the Plant Disease Survey. Dr. Edgerton, however, reported that this was not the first time he had observed it. The following year better and more abundant material was received through the Office of Foreign Plant Introduction. It had been obtained by H. Johansen during August, 1926, in the Plant Introduction Garden at Summit in the Canal Zone (Panama). Although two preliminary reports (4 and 5) concerning these two occurrences seem to be the only published accounts of Ustilaginoidea on Zea, the incidence of fungi of this genus on related hosts is not uncommon, especially in the Tropics.

The writers have not seen this disease in the field and, so, can describe it only from the dried specimens. The principal features thus far observed are the characteristic growths on the staminate inflorescence (Plate XIV, A and B) which, when mature, range from 4 to 15 mm. in diameter. These growths are very irregular in shape although, in general, they may be classed as subspherical. They are sclerotia of a fungus and not tumorous host tissue. Usually there is but one sclerotium on a spikelet although those on neighboring spikelets sometimes fuse to form compound sclerotia.

The surface of a mature sclerotium is much roughened, olive-green to almost black, and of a velvety appearance. The younger sclerotia are various tints of yellowish brown shading into green. When one cuts across a sclerotium the interior is seen to be made up for the most part of white, sclerotioid, fungous tissue including scattered host fragments. The surface layer is very different in texture and color from the interior tissue. The color shades from the white of the interior to yellow, then through brown to olive green and finally to the black of the surface. The extreme outer layer is found to be made up of a mass of mature spores with detached fertile hyphae. As the spores mature they become progressively darker, hence this surface is darker than the portion made up of younger spores and fertile hyphae directly beneath. This spore-bearing stratum covers the entire

periphery of the sclerotium to a depth of 50–200 μ. The fertile hyphae are closely packed at its base, becoming more loosely and irregularly arranged toward the exterior. They are septate, occasionally branched, yellowish green in color (by transmitted light) and 2–3 μ in diameter. The spores are borne on very short, sterigma-like projections from the walls of these hyphae and are spherical to ovate, 4–7 μ, usually 6 μ, in diameter, rarely larger. Immature spores are nearly smooth but with maturity the epispore is characterized by prominent pointed warts which, together with adhering matter, give an indistinct outline to the spore (Fig. 1).

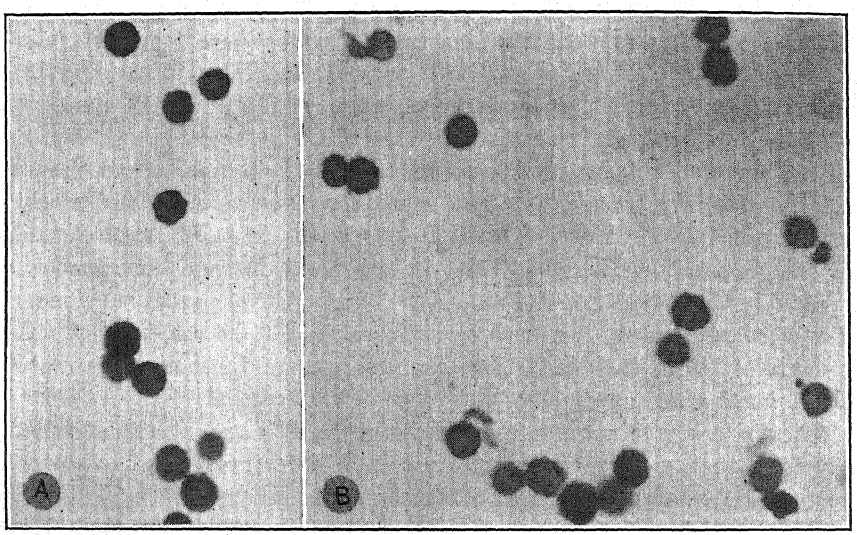


FIG. 1. Spores of Ustilaginoidea (×800), from (A) corn, Canal Zone; (B) rice, Crowley, Louisiana, 1921, collected by J. Mitchell Jenkins. (Photographed by J. F. Brewer.)

The identity of the species under consideration presents certain difficulties since the specific limitations and host relations are very imperfectly known. As far as specimens and records are available to the writers the hosts seem to be restricted to the family Gramineae. The fungus on corn appears to be distinct from the other species forming sclerotia exterior to the host tissues, but it is similar in measurable, microscopic characters to certain species having sclerotial development within the caryopsis. Of these latter it seems to resemble most closely the false smut of rice, *Ustilaginoidea virens* (Cke.) Tak., to which it is tentatively referred.

The false or green smut of rice is known to occur in all the great rice-growing areas except in Italy and Egypt. Butler (2 and 3) has discussed its distribution and significance. In the United States it has been recorded

with the evidence of specimens from Louisiana, only. There is, however, in the files of the Plant Disease Survey a report, unaccompanied by specimens, of its observation in Georgia, August 20, 1918, by J. B. Berry. In Louisiana it has been known since about 1900 (6, p. 505), when it was noticed as a curiosity by a few planters. Since that time it has been found occasionally but in no case has it been recorded as at all abundant.

The fungus and its effect on the rice plant have been described by various authors, notably by Butler (*l.c.*). On this host it develops within the grain, forming a sclerotium surrounded by a conidial layer. In its growth it bursts out between the closely applied glumes as a more or less spherical body (Plate XIV, C and D) which is orange tan to yellowish brown when young, becoming brownish green to almost black with age. In every detail so far observed, except for the somewhat smaller size of the sclerotium and its development mainly within the host tissue, *Ustilaginoidea virens* on rice resembles the form on corn.

Of the species of Ustilaginoidea on other hosts, those described with spores of similar dimensions are lacking in sclerotial development comparable with that discussed above and, until further evidence of relationship is available, may be omitted from consideration.

Ustilaginoidea virens has attracted only scant attention from the economic standpoint. One writer (8), however, ventures the opinion that the sclerotia may not be the sole evidence of injury to rice and that other losses such as spikelet sterility may be partly due to this cause. In the Philippines it is said (1 and 7) to cause an appreciable loss during some years of damp weather. In Louisiana it is not considered to be an economic factor.

On corn the disease probably will be even less injurious than on rice. As far as we know, it attacks only the tassels and results in little damage except a partial decrease in pollen production. There is a possibility that it may attack other parts of the plant, in which case the damage would naturally be more serious. It is an additional disease of one of our important crops and as such it should be watched. It is realized that owing to its superficial resemblance to corn smut it may easily be overlooked. When observations are made or specimens collected it is hoped that they may be reported to the Office of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

SUMMARY

A new disease of *Zea mays* is reported from Louisiana and the Canal Zone. Excrescences somewhat resembling those of common smut occur on the corn tassels. The causal fungus is an Ustilaginoidea and is tentatively referred to as *Ustilaginoidea virens* (Cke.) Tak.

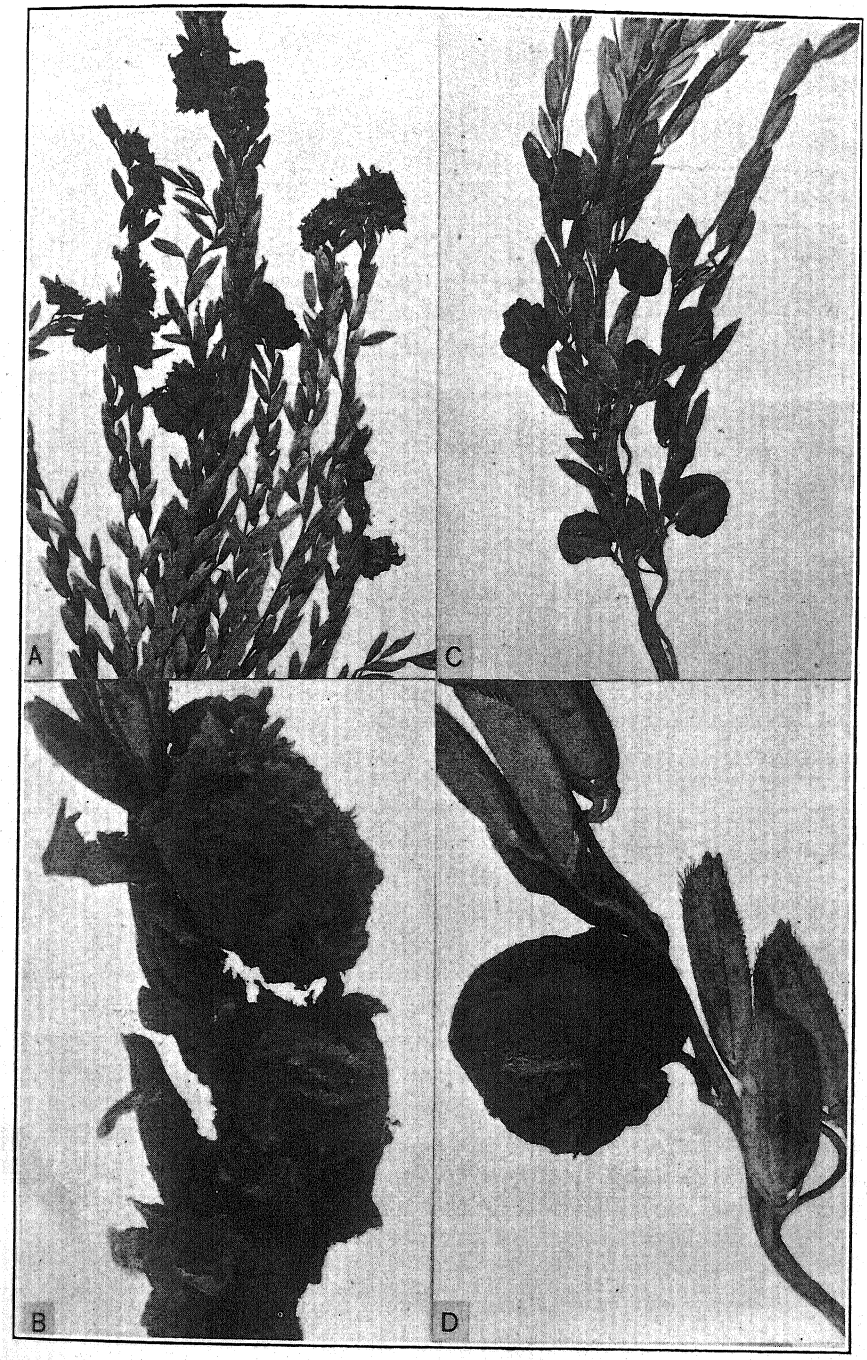
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EXPLANATION OF PLATE XIV

A. Ustilaginoidea on corn ×½ (material from Canal Zone). B. Detail of above ×12½. C. Ustilaginoidea on rice ×1 (material from Crowley, La.). D. Detail of above ×4. (Photographed by M. L. F. Foubert.)


A
C
B
D

A METHOD OF TESTING THE KEEPING QUALITY OF CERTAIN SMALL FRUITS

NEIL E. STEVENS

Breeding for disease resistance is almost a fad among plant pathologists and it is surely better, where practicable, to grow plants which will be largely free from disease than those which will produce profitable crops only with the aid of sprays or dusts. The interest in disease resistance, however, seems rarely to have extended even among the fruits, to breeding for resistance to decay in transit, on the market or in storage, that is, for keeping quality. Yet keeping quality has often been a prime factor in determining the commercial importance of a variety, as is evidenced by the Ben Davis apple, Elberta peach, Klondyke strawberry, and Howes cranberry.

The importance of keeping quality is perhaps widely recognized by breeders of small fruits and might very generally be considered as a factor in selecting varieties for propagation if some simple method for testing this character applicable to small quantities of fruit was generally available. The method described in the present paper has proved reasonably successful as a means of testing cranberries and blueberries.

METHOD

The method is simple. Pint samples from a given variety or lot are held in an electric incubator for one week at a temperature of 30 to 32° C. The fruit is then sorted. Cranberries are usually sorted into two lots, sound and rotten. Blueberries are usually sorted into four lots, perfect berries, shriveled (even slightly), soft, and moldy.

RESULTS

In the work with cranberries, attention was directed chiefly toward determining the relative keeping quality of different lots of standard varieties grown on various bogs through a series of years. For most varieties of cranberries when grown in Massachusetts or Wisconsin, the incubator test does seem to indicate, with a high degree of accuracy, the keeping quality of the different lots at ordinary storage and shipping temperatures. The chief exception seems to be the Howes which, in these States, is very generally affected by end rot caused by a fungus which will grow at temperatures only a little above 0° C. So far, we have not found this method so satisfactory for cranberries grown in New Jersey due, apparently, to the prevalence of high temperature fungi in that State.

For the past three years we have been using the same method as a means of testing the keeping quality of the new varieties of blueberries which are being planted commercially in New Jersey. As is generally known, these new varieties are either selected wild plants of *Vaccinium corymbosum* or crosses between plants of this species, or between this and other species, which have been developed and named by Dr. Frederick V. Coville. The past three years' work indicates that the varieties behave with a fair degree of consistency year after year and that their behavior in the incubator is a fairly reliable index of their behavior at common storage temperatures.

TABLE 1.—*Comparative keeping quality of blueberry varieties as indicated by average percentage of perfect berries*

One week at 30° C.		*Four weeks in cold storage*	
Rubel	20	Jersey	35
Jersey	18	Rubel	35
Pioneer	13	Grover	21
Grover	12	Pioneer	18
Cabot	9	Cabot	11

The work of the past year seems to show that for the five varieties that were tested in 1928 there is a fairly constant relation between the keeping quality of the different varieties at 30° C. and in commercial cold storage, as shown by table 1 which gives the average keeping quality throughout the season expressed as percentage of sound berries. This does not, of course, indicate the amount of edible berries, as even slightly withered berries are eliminated from the count, but the relative standing of varieties is not very different even if the total number of edible berries is considered, as is shown in table 2, in which perfect, shriveled, and spoiled berries were

TABLE 2.—*Comparative keeping quality of blueberry varieties according to a weighted scale*[1]

One week at 30° C.		*Four weeks in commercial cold storage*	
Jersey	60	Jersey	72
Rubel	41	Rubel	67
Pioneer	24	Grover	65
Grover	20	Pioneer	53
Cabot	17	Cabot	36

[1] The values in this table were obtained by assigning a value of 1 to each perfect berry, a value of 2/3 to each shriveled berry and −1 to the soft or moldy berries. It makes little difference in the relative standing of the varieties what values are chosen.

all considered according to a weighted scale. It will be noted that Rubel and Jersey occupy a high position, Grover and Pioneer are intermediate, while Cabot is lowest in all the columns.

DISCUSSION

It is, of course, not to be expected that this close correspondence in behavior at high and low temperatures will be found among all small fruits or that it will necessarily hold for all the new varieties of blueberries that are constantly being developed. Overholser[2] has recently called attention to the fact that "the better the intrinsic keeping quality at room temperatures, of the species of fruit stored the greater the proportional relative delay in senescence brought about by cold temperatures." He further points out "that certain varieties within a given species kept relatively long at 18° to 24° C. and comparatively short periods of time at 0° C. Furthermore, with other varieties of the same species the reverse was true."

In spite of this undoubted difference between the varieties of some species, there may be sufficient correlation in the behavior of others at high and at lower temperatures for one to serve as a working index of the other. At least the apparent success of the method in this case seems to justify its further use and study.

To consider keeping quality as an important character in breeding does not mean a neglect of eating quality. It will undoubtedly be possible to combine in the same berry high flavor and good keeping quality. In the past, by a process of natural or commercial selection, those varieties have been most largely planted which showed good keeping quality regardless of their eating quality. The result has often been extensive plantings of varieties of distinctly inferior eating quality. An example of this is the Klondyke strawberry which occupies about one-fourth of the commercial acreage of strawberries in the country. This variety, incidentally, is said to have been developed as a result of selection for keeping quality. The Bureau of Plant Industry already has in its breeding plots at Bell, Md., varieties far superior to it in eating quality which seem, from our preliminary tests, to be superior in keeping quality also.

As regards the blueberry, the possibility of putting on the market a variety which did not possess good eating quality has never been considered. All the varieties used in these tests are of high, although not equal, eating quality. We are merely trying to determine which of these unusual fruits has the best keeping quality. The work of testing varieties of blueberries will be continued, and we plan to try a modification of this method on strawberries.

[2] Overholser, E. L. Cold storage versus room temperatures on keeping qualities of fruits. Science 66: 660–661. 1927.

SUMMARY

Holding pint samples of blueberries and cranberries at 30° C. for one week apparently gives a fairly reliable index of their keeping quality at lower temperatures.

In view of the recognized importance of keeping quality in determining commercial importance of varieties of fruits, it is suggested that this method is worthy of further trial by breeders of small fruits.

BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

BLUE-STAINING FUNGI FOUND IN THE UNITED STATES

CAROLINE T. RUMBOLD

This article presents the results of an investigation conducted by the Bureau of Plant Industry in cooperation with the Forest Products Laboratory to obtain information on the species and distribution of blue-staining fungi infecting the sapwood of hardwoods and of softwoods in the continental United States and in Canada. These fungi appear to divide themselves into three general groups which are found respectively in three different places: In the forest, on green wood in the lumber yard, and on wood that is old or that has been heated.

FUNGI IN THE FOREST

Although Ceratostomella and Graphium appear to be the most common of the staining fungi in every part of the country, they are found in the greatest variety of forms in the forests. Here also are some Pyrenomycetes, pycnidial forms, perhaps of Valsa, and some molds, many of them non-staining, among which Trichoderma is important.

FUNGI ON GREEN LUMBER

The flora developing in the lumber yard on the freshly sawed boards seasoning in the stacks is less varied than that in the forest. Ceratostomella and Graphium are still the fungi commonly found, but the number of forms has decreased. *Ceratostomella pilifera,* the blue-staining fungus common in lumber yards in all parts of the United States, is the important form; *Graphium rigidum,* the gray or bluish gray-staining one, is frequently a companion of it. These fungi are hardy and rapid growers. Other varieties of Ceratostomella and Graphium also occur on such lumber; and, in addition, there are some fungi of the Valsa-like group and some molds, but they are not the important stainers of the freshly cut and seasoning boards.

FUNGI ON LUMBER THAT HAS BEEN HEATED OR DRIED

After the sapwood boards have been steamed or kiln dried, or when they are old, the flora living in the blue-stained wood is still different. It consists now of the Valsa relatives, molds, and a black mycelium, which has not yet fruited in culture. Ceratostomella and Graphium do not infect such boards. Wood rots generally are present in the old wood, but they do not stain the wood blue. Also most of the molds found have colorless mycelium instead of brown and probably do not stain.

TABLE 1.—*Fungi cultivated from blue-stained sapwood collected in the United States and Canada*

Host genus	Number of States and Provinces represented	Total number of specimens examined	Number of specimens that yielded Ceratostomella (C) or Graphium (G)											Number of specimens yielding				Other fungi
			1C	2C	3C	1G	1C 1G	2C 1G	1C 2G	3C 1G	2C 2G	4C 1G	3C 2G	*Valsa* sp. (pycnidial stage)	Sterile black fungus	*Trichoderma lignorum*	*Endoconidiophora* sp.	
								Fresh specimens from the forest										
rcus	4	31	1	2	1	3	8	6	1	2	1	1		3	1			Atractium, Stemonites, Pestalozzia
us	5	23	13	1		3	1							1			3	*Dematium pullulens*
odendron	3	9	1	1	1	2		3		1								
oria	5	5			1		2			1			1					
uidambar	2	5		1	1		2	1										
us	2	4					1	2		1								
culus	2	2			1					1								
a	2	2	1					1										
ga	1	2				1	1											
ionia	1	1												1				
anea	1	2					1	1										
litsia	1	1						1						1				
ulus	1	1							1									
ius	1	1					1											
								Specimens from stacks of green lumber in yards										
us	6	23	13			3	3								3			Stachybotrys
a	3	6				6												
idambar	2	4	1			1	1									1	1	
nolia	1	4	3				1								1			
	1	4												2	2			
a	1	3				3												
la	1	2				1	1								1			
cus	1	1												2 spp.				
	1	1	1															
								Specimens of heated and of old wood										
s	6	10												1	1	2		3 Wood rots,[1] Stemonitis, *Penicillium pinopholum*
idambar	2	5												2	2	1		Alternaria, *Monilia sitophila*, 2 Penicillium
cus	1	1													1			

[1] These wood-rotting fungi were in the blue-stained wood of logs that had been lying in the forest 3 years. It is probable the blue-staining i had died.

OBSERVATIONS

Table 1 shows the general situation. The fungi listed were growing in blue- or gray-stained sapwood; an explanation of how they were cultivated from the wood will make the data in the table clearer. First, they were obtained by gouging out a sliver of the discolored wood from the interior of the specimen with a quarter-inch-wide forceps-chisel. The sliver was always taken from the inside of the board, chip, or branch, in order to avoid picking up fungus spores that perchance might have fallen on the surface of the specimen without being implicated in its stained condition. After being thus cut out the slivers were transferred to malt-agar slants, where they had an opportunity to grow from the wood to the agar. Naturally, only such fungi were cultivated as were vigorous enough to grow out on the agar. Doubtless many species and numerous varieties were suppressed by more vigorous ones and hence were overlooked when retransfers were made. Still, it is a surprising fact that as many as three strains of Ceratostomella and two of Graphium have grown from a sliver of wood, no more than a quarter-inch wide, taken from a chip picked up in the forest.

"Ceratostomella and Graphium" in the heading of the fourth column of the table means that Ceratostomella and Graphium are separate fungi. In other words, the Graphiums in general are not imperfect stages of Ceratostomella.

As a rule, the specimens from the forest were infected with more than one strain of fungus. The pines were exceptions to this rule except in two cases. One of the reasons for this is the fact that 7 of the 23 specimens of pine listed had been taken from trees infested with the bark beetle Dendroctonus and 8 with the beetle Ips. Ordinarily, the freshly bored canals of these beetles are infected with only a single blue-stain fungus, specific for Dendroctonus, or, another, specific for Ips. In the case of one of the pine specimens infested with Dendroctonus, however, the brood of beetles had died in the trees. When cultured the blue-stained wood from this specimen produced *Ceratostomella pilifera* besides the fungus commonly associated with the bark borer. The other example of a condition unusual for the forest pines examined was a specimen infested with Ips, which produced *Graphium penicillioides* in addition to the Ceratostomella found with Ips.

OFFICE OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
WASHINGTON, D. C.

ANOTHER ANTHRACNOSE OF RASPBERRY

S. M. ZELLER

During the last five years the writer has collected from various places in western Oregon and Washington samples of a Gloeosporium which produces an anthracnose of the wild raspberry (*Rubus leucodermis* Douglas), in particular. Rather infrequently the disease has been found on cultivated varieties of black raspberry and the St. Regis variety of red raspberry. So far as has been observed this occurs only when the cultivated varieties have been planted near infected *R. leucodermis* in neighboring wood-lots. The known distribution of the disease is from Douglas County, Oregon, to British Columbia, but perhaps it is more widely distributed over the range of the wild host, *R. leucodermis*.

The causal organism answers in every morphological character the description of *Gloeosporium allantosporum* Fautrey. Fautrey says this species differs from *G. vincetoxici* Fautrey in spore size, but otherwise is similar except in host preference. Perhaps this is a variety of the latter species but we prefer to refer to it as *G. allantosporum*. The name is applicable to Pacific Coast material, the spores of which are characteristically allantoid and measure 16–20 x 3 μ.

DESCRIPTION OF LESIONS

So far as known the disease is confined to the canes. The spots on the canes are long and narrowly elliptical. First infections of new canes take place with the first rains in the fall, but later infection may occur well into the spring. The lesions first appear as longitudinal marks, about ¼-inch in length and as broad as ordinary pencil marks. These long, narrow early indications of infection in the fall are greenish-brown with a water-soaked appearance. These enlarge very slowly and become pale-yellowish and then as the canes become dormant in the late fall, the lesions turn white and ashen. The lesions are not so deeply sunken as in the case of the well-known anthracnose of black raspberries caused by *Plectodiscella veneta* Burk., due perhaps to the fact that the vascular and more firmly organized elements of the bark of the host are rather well matured before infection takes place. Infection involves the epidermis and outer layers of cortex only. Lesion margins become reddish brown, forming a considerable "halo" around the lesions in most cases. The mature lesions are long and narrow in contrast to the very nearly circular ovals in the anthracnose caused by *P. veneta*. Lesions produced by the latter often occur on the same canes along with those produced by *Gloeosporium allantosporum*.

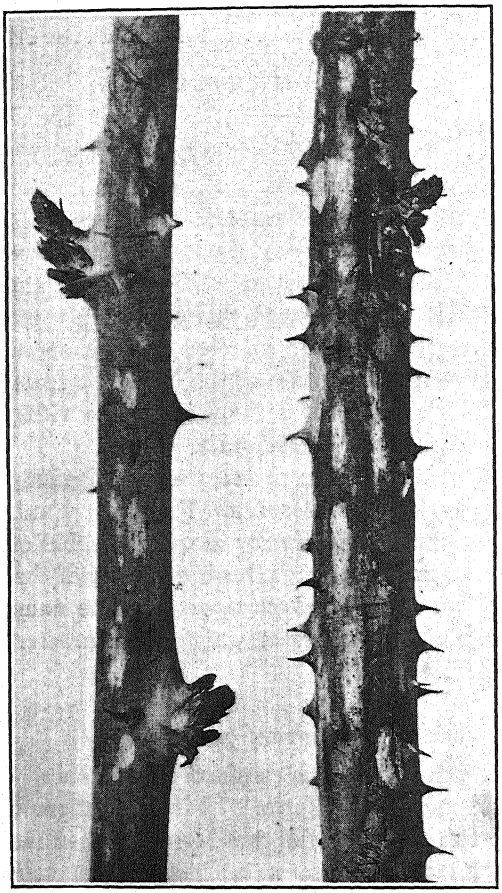


FIG. 1. Lesions produced by *Gloeosporium allantosporum* on canes of *Rubus leucodermis*. The definite, elliptical lesion on the lower part of the right-hand cane is produced by *Plectodiscella veneta*.

The lesions do not show any indications of fruiting bodies the first season, but early in the spring from February until June and again in the fall, the acervuli appear as tiny black dots over the surface. The cane lesions do not materially differ in appearance from those produced by *Rhabdospora rubi* Ellis.[1] The acervuli are often so close together that the canes are practically girdled. In such cases, however, the disease is not so serious as in the well-known anthracnose because the lesions are not so deep-seated but extend only a short distance under the epidermis.

[1] Zeller, S. M. Contribution to our knowledge of Oregon fungi. Mycologia 21: 97–111. 1929. (See Fig. 2, p. 109.)

CONTROL MEASURES

In the fall of 1926 and spring of 1927, it was convenient to apply sprays to some of the western wild raspberries in a locality where this anthracnose was very bad. These two sets of spraying tests were applied in the following manner. In 1926 in the first trials Bordeaux 4–4–50 was applied before the fall rains began and in the spring of 1927 in the second spray test two applications of Bordeaux 4–4–50 were given, one when the canes were 18 inches high and another when about 25 inches high. In the first test the plants so sprayed had no anthracnose in the spring of 1927. In the case where one application was applied when the canes were 18 inches high and again when 25 inches high there were present in the spring of 1928 eighty-seven per cent as many lesions caused by *G. allantosporum* as on neighboring plants which were left unsprayed. In these cases, however, the lower 18 inches of the canes had decidedly fewer lesions than the portion higher up. The spray applied just before the discharge of spores in the fall is effective in controlling new infections on the canes grown during the current season. This information will perhaps help in the control of this disease in black raspberry plantings near open wood-lots where infected plants of wild black raspberry are present.

SUMMARY

In western Oregon and Washington, an anthracnose caused by *Gloeosporium allantosporum* occurs on the wild black raspberry (*Rubus leucodermis*) and on cultivated varieties of black raspberry and the St. Regis variety of red raspberry when the latter are planted near the wild host harboring the disease. This paper describes the disease briefly. Bordeaux 4–4–50 applied just before the discharge of spores in the fall controls the disease on canes grown during the current season.

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PHYTOPATHOLOGICAL NOTES

A mutation in Gloeosporium. While a number of petri-dish cultures of Gloeosporium, isolated from a lemon, were being grown in the Mycology Laboratory, one culture behaved differently from the rest. Instead of producing the usual dark appearance (Fig. 1, 1) due to an abundance of acervuli, it had in parts the characteristic dark appearance (Fig. 1, 2A)

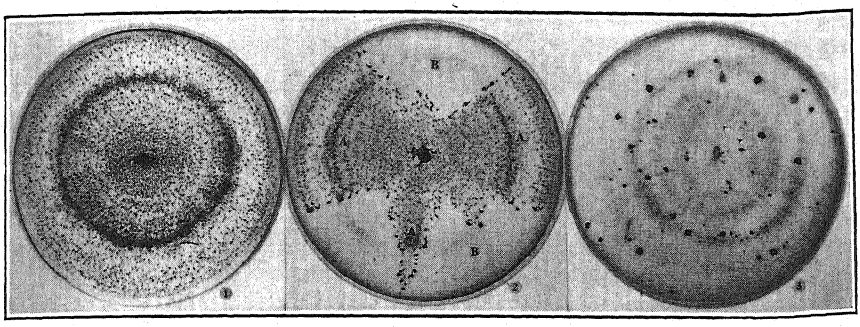


FIG. 1. 1. Gloeosporium culture from 2A, the usual appearance.
2. Culture from a plate similar to that in 1, showing the usual type of growth in A and the new form in B.
3. Culture from 2B showing the new form of growth as contrasted with the old form in 1.

and in others a lighter appearance (Fig. 1, 2B) due to the presence of only a few scattered orange and black acervuli. Subsequent cultures made from the dark (A) and light (B) segments showed that the respective transfers remained constant for the characteristics present in the original segments (Fig. 1, 1 and 3). It would appear then that a sudden and permanent variation or mutation had occurred. No variation was observed in conidia form.—JACQ. P. F. SELLSCHOP, Department of Botany, University of Illinois, Urbana, Ill.

A note on the longevity of the bean mosaic virus. A collection of 150 varieties of domestic and foreign beans made in 1897 by the Horticulturist of the Idaho Agricultural Experiment Station was recently turned over to the Department of Plant Pathology. These varieties were stored in 6″ glass vials and bore the date 1897. Horticultural records show that they were planted in 1898 and 1899, but there is no evidence of their having been planted since that time. In attempts to germinate seed of a large number of these varieties, only the following were viable: New German Wax Flag; Guatemala Red (native variety); a strain of Frijol bayo from Matamoras, Mexico; and an unnamed black wax variety, No. 24.

On February 20, 1929, from 10 to 25 seeds of each of the above varieties were planted in the greenhouse. With the exception of the black wax

variety (No. 24), all of the plants obtained were apparently free from disease. Only two black wax plants were secured, but one of these exhibited mosaic mottling in the two seed leaves as soon as the plant emerged. As new leaves appeared, unmistakable mosaic mottling also could be seen in them. There was no curling of the leaf margins associated with the mottling, but the leaves were considerably narrower and longer than those of the healthy plant.

On March 22, several of the mottled leaves were removed from the plant and thoroughly macerated in a sterile mortar. This material then was used to inoculate 12 healthy Great Northern bean plants. Symptoms of mosaic typical of the Great Northern variety had appeared on six of the plants by the 3rd of April. A few days later, 10 plants showed mosaic symptoms, and thus furnished conclusive evidence that the bean mosaic virus had remained viable within the seed for a period of at least thirty years.—W. H. PIERCE and C. W. HUNGERFORD, Department of Plant Pathology, University of Idaho, Moscow, Idaho.

Summer meetings of the Botanical Society of America. In accordance with the sentiment expressed by the members in a recent poll, The Botanical Society of America will conduct two summer meetings during the present year. One of these will be held at Dartmouth College, Hanover, N. H., beginning on the evening of June 25 and extending through June 28. The other will be held at the mountain camp of the University of Wyoming from July 31 to August 3.

These meetings are not to be patterned after the regular winter meetings, where emphasis is placed upon the presentation of the results of research in formal papers. They will be characterized rather by informality, the time being devoted to field excursions and round-table discussions. Everything will be done to promote that personal association out-of-doors which is necessarily lacking at the more formal and crowded meetings.

At the Dartmouth meeting it is planned to have an informal reception and an address of general interest on the opening evening, a number of morning round-table discussions of special topics, two afternoon field trips, one all-day excursion, and an open discussion of botanical teaching methods.

Registration for the Wyoming meeting will occur at Laramie on the afternoon of July 31; this will be followed by an informal dinner and reception. On the following morning those in attendance will be taken by automobile to the mountain camp, where three days will be occupied by field excursions and round-table discussions.

The Chairman of the Pacific Section of the Society has invited the members to consider the annual meeting of the Section, to be held in Berkeley on June 20 and 21, as another summer meeting of the Society.

A cordial invitation to attend the meetings is extended to botanists who are not members of the Society, and especially to those who may be visiting this country from abroad.

A circular containing more detailed information was mailed to every member of the Society in May. Inquiries may be addressed to the undersigned or to the chairmen of the local committees: Prof. Aven Nelson, University of Wyoming, Laramie, Wyoming, and Prof. A. H. Chivers, Dartmouth College, Hanover, N. H.—L. W. SHARP, *Chairman of the Organizing Committee*, Cornell University, Ithaca, N. Y.

INTERNATIONAL CONFERENCE FOR PHYTOPATHOLOGY AND ECONOMIC ENTOMOLOGY—HOLLAND, 1929

SECOND NOTICE ON ERIKSSON PRIZES

The International Committee for Phytopathology and Economic Entomology beg to announce that two prizes are hereby offered for the two best Memoirs, giving an account of new and original work on the two following subjects respectively:

(1) Investigations on Rust (Uredineae) Diseases of Cereals (Wheat, Oats, Barley or Rye).

(2) Investigations on the rôle played by insects or other invertebrates in the transmission or initiation of Virus Disease in Plants.

The value of each prize will be 1,000 Swedish crowns.

Competitors may be of any nationality.

Three typewritten copies of each Memoir must be submitted. They may be written in any one of the three languages, English, French or German.

Memoirs must reach the Secretary on or before May 1st, 1930.

The author's name must not appear on the Memoir itself, but each Memoir must be marked with a pseudonym or a motto and the full name and address of the author must accompany the Memoir, being enclosed in a sealed envelope bearing on its outside the same pseudonym or motto as is given on the Memoir.

The adjudication of the rust prize will rest with a jury, consisting of Professor Dr. Jacob Eriksson, Professor Dr. E. C. Stakman and Professor Et. Foëx.

The adjudication of the virus prize will rest with a jury, consisting of Professor Dr. H. M. Quanjer, Dr. A. D. Imms and Dr. L. O. Kunkel.

The decisions of these juries will be final and will be announced at the Fifth International Botanical Congress, to be held in Cambridge (England) from August 16th to 30th, 1930. The copyright of the prize Memoirs will become the property of the Committee, who will endeavor to secure publication of them in a suitable existing periodical or, failing that, procure

publication in some other way. Other Memoirs will be returned to their respective authors.

The Committee reserve the right to withhold the prizes should none of the Memoirs submitted be deemed of sufficient merit by the respective juries.

Further particulars, if required, may be obtained on application to the Secretary.

For the International Committee for Phytopathology and Economic Entomology.

T. A. C. SCHOEVERS, *Secretary*,
Nassauweg 28, Wageningen, Holland.

Notice of Summer Meeting. The summer meeting of the American Phytopathological Society will be held in conjunction with the regular annual meeting of the Southern Division, July 28–31, 1929. The committee in charge: R. F. Poole, Chairman; G. W. Fant, Secretary; S. G. Lehman, S. A. Wingard, and G. M. Armstrong, has arranged a three days' meeting and tour starting at the College of Agriculture at Raleigh, thence northward to Oxford, then southward to Duke University at Durham, the University of North Carolina at Chapel Hill, Sanford, Southern Pines, Pinehurst, and Hamlet. This itinerary will take the group through some of the important educational centers and famous winter resorts as well as through some of the most intensive and interesting crop sections. Ample opportunity will be given to observe and study diseases of crops such as tobacco, sweet potatoes, cotton, peach, soybeans, and dewberries. The cooperation of the horticulturists and agronomists of the Agricultural College has been obtained. Demonstrations and experiments on disease control have been arranged at various points along the line of travel. These attractions, coupled with the fact that the spring and early summer weather has been favorable for plant-disease development, should insure a successful meeting.

Those who expect to attend should communicate with the chairman of the committee at the North Carolina State College, Raleigh, as soon as possible.—COMMITTEE ON ARRANGEMENTS.

Lyman Memorial Fund. The total of the Lyman Memorial Fund for phytopathological publications already is about $1,000.00. This includes cash on hand and pledges. Individual cash donations range from $1.00 to $50.00, and the pledges range from $1.00 a year for a short period of time to $25.00 a year for an indefinite period. It appears that the project will be a success. It is hoped that the endowment fund will continue to grow.

In addition to the Lyman Memorial Fund, the National Research Council has generously given $500.00 to be used before July 1, 1929.

BOOK REVIEW

Handbook of Microscopical Technique. For workers in both animal and plant tissues. Consisting of contributions from 24 specialists, edited by C. E. McClung. *XIV + 495 pp. 43 figs.* Price $8.00. Paul B. Hoeber, Inc.: New York, 1929.—The following statement is from the preface: "In two previous texts, General Cytology and Special Cytology [ed. by E. V. Cowdry] experts in various fields of biology have presented the results of their investigations. In the present work, which extends the series, the *methods* involved in these and similar studies are given. There are two general needs to be met by a work of this sort. The first is that of the unexperienced worker who requires specific directions which he may apply with confidence to the general run of material, with the expectation of getting desired results. (Part I . . . outlines such standard methods.) The other requirement is that of the experienced investigator who seeks the latest approved methods for the accomplishment of special technical results. In order to meet such a need as this, involving, as it does, the intimate knowledge of varied and often involved processes, the method of cooperation employed in the above-mentioned books is utilized. Workers highly experienced in the use of specialized methods describe this in detail."

A partial list of the chapter headings and the respective authors is: Section and non-section methods of preparing microscopical slides, C. E. McClung; Microdissection, microinjection, Robert Chambers; Vital stains, Nathan Chandler Foote; Supravital stains, Florence R. Sabin; Bacteriological methods, H. J. Conn, F. B. Mallory, and Frederic Parker, Jr.; General botanical microtechnique, William Randolph Taylor; Protozoological methods, D. H. Wenrich; Fixation and fixatives, C. E. McClung and Ezra Allen; Stains and staining, C. E. McClung and H. J. Conn.

The remaining chapters have special reference to zoological and medical cytology and histology, though among the many details presented doubtless much of value to the special worker in other fields also is to be found.

The discussion of non-section methods, though written from the viewpoint of a zoologist, should be of considerable value to workers in plant tissues, among whom a tendency to rely perhaps too much on sectioning methods has been manifest. Of special importance is the discussion of methods for the study of fresh material, including microdissection, microinjection, vital staining and intracellular indicators—in short the "newer" technique. Certain of these methods are already in use in special studies of plant cells as physical properties of cell constituents, nature of inclusions; others can be adapted.

In the chapter on botanical microtechnique, the treatment of special subjects, such as fungi and the differentiation of fungus mycelium from

host tissue, is somewhat less extensive than in a book particularly devoted to the botanical field, as Chamberlain's Methods in Plant Histology, but phytopathologists might wish for more emphasis on this subject in either work.

Throughout the book particular emphasis is placed on the rationale of technical methods. This feature should further promote the proper application of the details and authoritative exposition of processes.—FREEMAN WEISS, Bureau of Plant Industry, Washington, D. C.

PHYTOPATHOLOGY

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A DESTRUCTIVE ROOT DISEASE OF PINEAPPLES AND OTHER PLANTS DUE TO TYLENCHUS BRACHYURUS, N. SP.

G. H. GODFREY

During the past year, considerable study has been devoted to a pineapple-root disease, widespread in its occurrence in the Hawaiian Islands. This disease is characterized by brown lesions, and in more severe cases by the death of many of the branch rootlets and even of some of the main roots. The lesions are caused by a species of Tylenchus, apparently different from any yet described. Often the very common nematode root knot is accompanied by this Tylenchus, and under the combined attack of the two organisms, *Heterodera* (Caconema) *radicicola* and **Tylenchus brachyurus**, n. sp., extreme root destruction sometimes results. This paper deals with the detailed description of the disease, with its distribution in so far as it is known, with experimental inoculations, with the description and identification of the nematode, with its relations to the host tissues, and with observations on the relationship of the nematode to its environment.

DESCRIPTION OF THE DISEASE

Plate XV depicts the characteristic symptoms of the disease. The root lesions which characterize it in the early stages are irregularly elongate argus brown spots. They are somewhat lighter or more dilute in color when very young (*G*), darker and deeper in color as they grow older (*A* and *B*). They show sharp contrasts in surface colors, the edge is sharply delimited from the normal tissues on the side or end, and the surface of the lesion itself is sometimes mottled or striped with darker and lighter browns. The outlines of the ends and sides of a lesion are determined by the outlines of individual dead and discolored epidermal cells. This is shown by Plate XV, *I*. Plate XV, *F*, shows that a single lesion penetrates into the root irregularly, being limited at its greatest depth by the stele. The lesions are not sunken. Their appearance, as illustrated, is very characteristic. They are readily distinguishable from spots and lesions produced by other organisms.

Few or many lesions may appear on a single root, depending upon the degree of infestation of the soil. When many are present they run to-

gether and darken the entire root. Plate XV, *A* and *B*, shows both single lesions and united lesions. The latter result in serious damage. If such regions are at the tip of a root, they stop further growth and the root tip breaks down. Thus large numbers of nematodes are released into the soil to attack such branch roots as may develop. If, as they often are, these heavily infested regions are well up on a young root, the entire cortical region for some distance is destroyed, the vascular system is ultimately

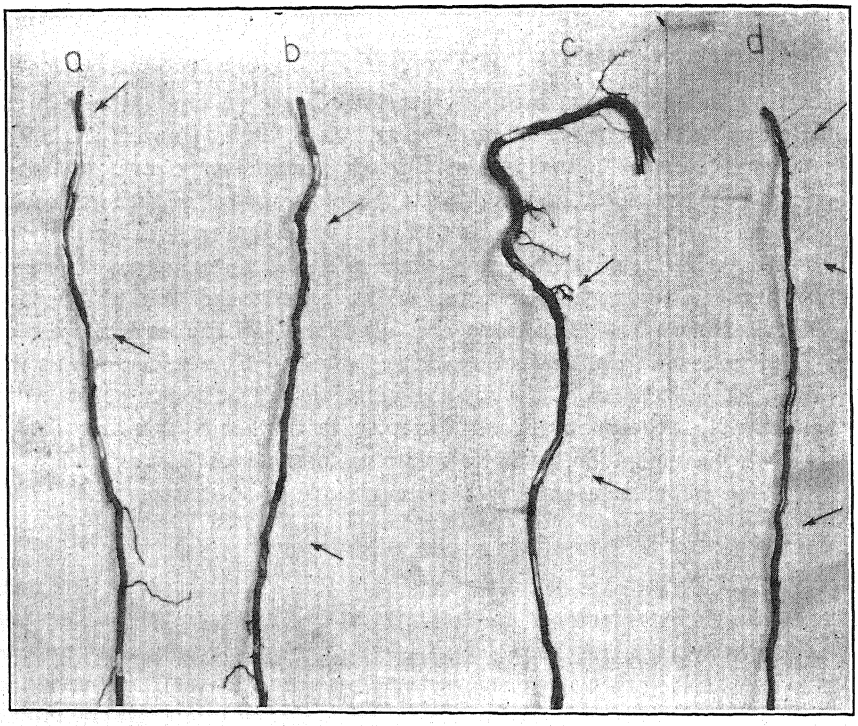


FIG. 1. Well developed pineapple roots, 1½ to 2 feet long, with the cortex completely invaded by Tylenchus in the regions whose limits are indicated by arrows. The root to the left is already dead in this region, which means that the root is doomed. In the other roots the stele is still alive but will probably die shortly. The white areas on the middle portions of the roots are cuts, to show that these regions are normal.

killed, and the entire root below this point, of course, dies. Figure 1 shows a series of roots from a single plant, heavily infested in the upper portions for greater or less distances, the lower portions being relatively free from invasion but doomed, nevertheless. Plate XV, *E*, shows a section of one of the well advanced heavily infested regions, with complete deterioration of the cortex, and evident injury in the stele.

Heavy infestation of the soil results often in the killing of a large number of tender branch rootlets. Plate XV, *A* and *B*, shows the typical appearance of such roots, invaded by the nematodes and still living.

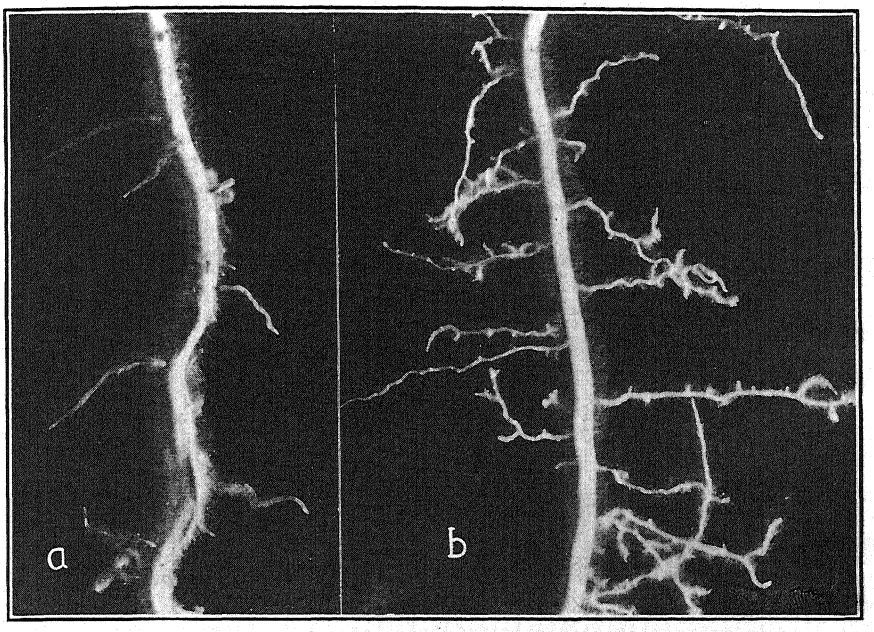


FIG. 2. Differences in branching, and therefore in water-absorbing surface, between a Tylenchus infested (*a*) and a healthy root (*b*). Many of the branch roots in (*a*) have been killed; others have been invaded and checked in growth, and would probably have died.

Figure 2 shows in *a*, a root with branches greatly depleted through invasion by the Tylenchus, and, in *b*, a normal root and branches for comparison. Obviously, the feeding potentialities of the root system may be greatly handicapped by this organism. If the Tylenchus is present in pineapple fields infested by the root-knot nematode, it almost invariably accompanies that nematode in the galls. Its presence can be established macroscopically by cutting or breaking open a firm gall and looking for the brownish color, which is a sure indication. Plate XV, *C* and *D*, shows the typical appearance of a gall beginning to break down because of the heavy Tylenchus infestation. Figure 3 is another root tip suffering from both Heterodera and Tylenchus infestation. As shown by these pictures, the Tylenchus occurs mostly in the outer portions of the root, the Heterodera being established normally deeper in the tissues, with its head end adjacent to the pericycle of the stele. An explanation for the frequent occurrence of the

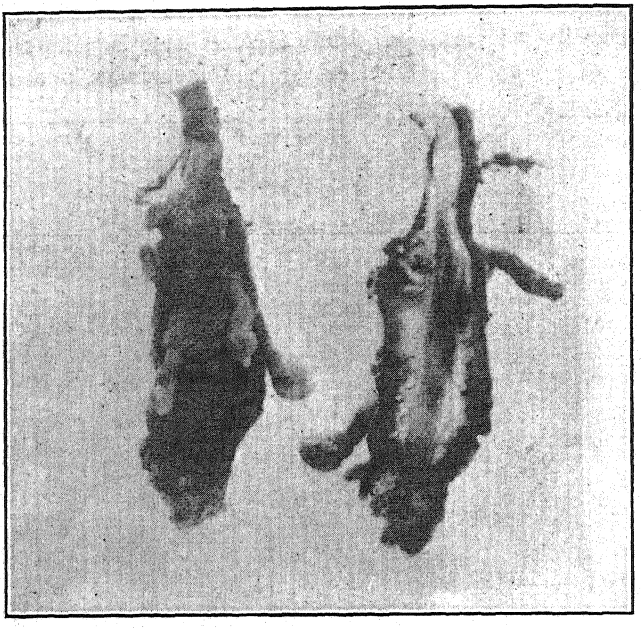


FIG. 3. Heterodera root gall in pineapple, invaded by Tylenchus as well. The picture to the left is a surface view, that to the right a longitudinal section. Such a root, checked in its forward growth by the root knot nematode, becomes an easy prey to the Tylenchus if that organism is moderately abundant in the soil. The cortex and tip of the main root are completely invaded by the Tylenchus, as are also the branch roots. Individuals of the root knot nematode may be seen with their head ends at the border of the stele.

Tylenchus in Heterodera galls is included in the section, "Relation of the nematode to the host tissues."

HOST RANGE, AND SYMPTOMS ON PLANTS OTHER THAN PINEAPPLE

The symptoms on other plants are very similar to those on pineapple. In certain phases of the experimental work during the past year, cowpeas (*Vigna sinensis*) and soybeans (*Glycine hispida*) have been used as indicator crops to show the presence of the root-knot nematode in the soil without disturbing the pineapple plants. Both of these plants have served also as very effective Tylenchus indicators. Infection shows up as brown lesions on the roots and rootlets. Plate XV, *H*, *I*, *J*, and *K*, shows typical symptoms on cowpea roots. *I* is the same lesion as the lower one on *H*, considerably more enlarged. The young roots of these plants are smaller than those of the pineapple, and are structurally more delicate. Consequently, the killing of roots is likely to be more pronounced. Figure 4 shows a

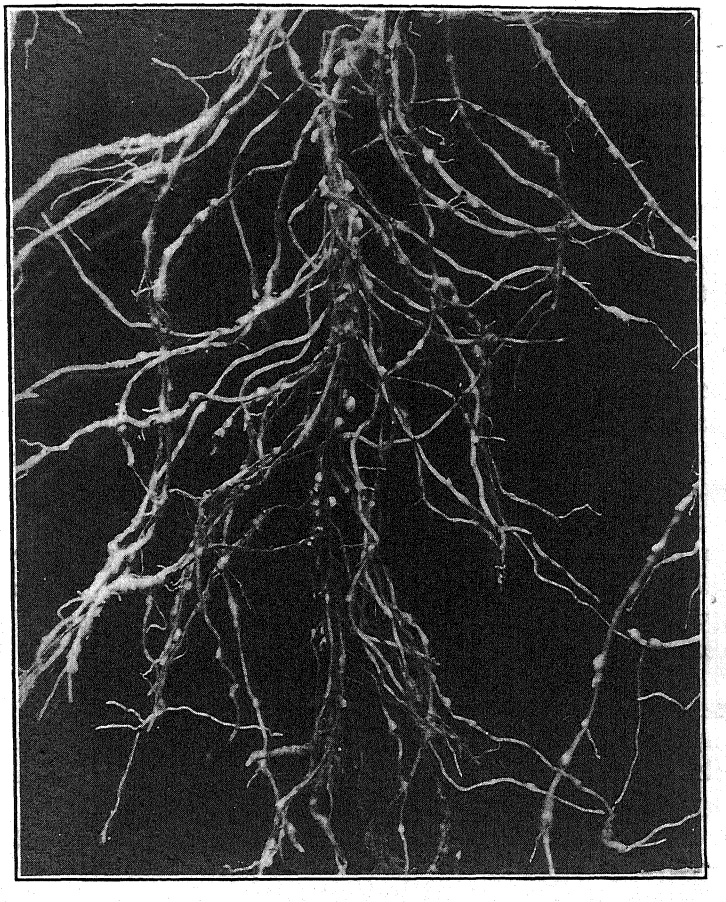


FIG. 4. Heavy Tylenchus infestation in a cowpea root growing under otherwise good conditions. Note the brown striped appearance of the tap root throughout its length. Many of the branch roots likewise show lesions. The tip of the main root has been killed. The tissues were heavily invaded by *Tylenchus brachyurus*.

much branched cowpea root, very heavily infested with Tylenchus. The tip of the main root is wholly decayed, and the remaining discolored portion was found to be heavily populated with Tylenchus in all stages of development.

In tomato, likewise, as used as an indicator crop, similar lesions are to be seen but their scarcity indicates a decided preference of the nematode for other plants. No extreme infestation was found on any weeds, but typical lesions containing the Tylenchus were found on the red-flower "pualele"[1] (*Emilia sonchifolia*) and a few others. Dr. Frederick Muir

[1] Hawaiian for "flying seed."

informs me (personal communication) that he has seen this same form in sugar-cane roots, but never very extensively.

Since the choice of rotation crops will be affected materially by the finding of this nematode, it is expected that a great deal of study will be devoted to its host range.

DISTRIBUTION OF THE DISEASE

The Tylenchus root lesion has been found well distributed over the pineapple plantations in the Hawaiian Islands. The first finding was in the roots of plants from a field on Kauai, in August, 1926. Susbsequent examinations of plants from this same location have shown it to be abundant in that particular field. The gradually accumulating evidence from survey records show it to be present in greater or less seriousness in practically every section of the island of Oahu; and from some parts of the islands, Maui, Molokai and Lanai. Many fields appear to be entirely free from infestation. A complete survey has by no means been made.

Interesting observations have been made from time to time on its occurrence in relation to the root knot nematode. In by far the majority of cases observed both occur together. In many locations the root knot nematode occurs without the Tylenchus. And in some places, root knot has been absent and the Tylenchus has been present.

EXPERIMENTAL

Many inoculation studies were made and it was soon learned that **Tylenchus brachyurus**, n. sp., is capable of bringing about rapid injury to young, actively growing roots of pineapples and other plants. At first a water suspension of adults, larvae, and eggs dissected out from the roots of infested plants from the field, was used as inoculum. This was poured over the young root tips, as they appeared under the glass in our root study boxes (5).[2] In four days primary discoloration was evident. In six days, a lesion such as the smaller ones shown in Plate XV, *G*, was evident. A few days later, lesions were prominent on all inoculated roots. Dissection of such lesions showed that both adults and larvae penetrated the roots. In one instance, eight adults only were placed in a drop of water on a root tip and a lesion developed. A month later, other lesions were evident lower down in the same roots, showing that young had already hatched and the progeny had migrated to growing tips.

Infected roots, cut from plants and placed immediately below developing roots in root-study boxes resulted in heavy infection. Pineapple roots

[2] A complete description of the root-observation boxes used for this type of work has been prepared by Dr. A. L. Dean, Director of the Pineapple Experiment Station of the Association of Hawaiian Pineapple Canners. Figure 5 is a photograph of one of the smaller type of boxes, and shows the excellent availability of roots for inoculations.

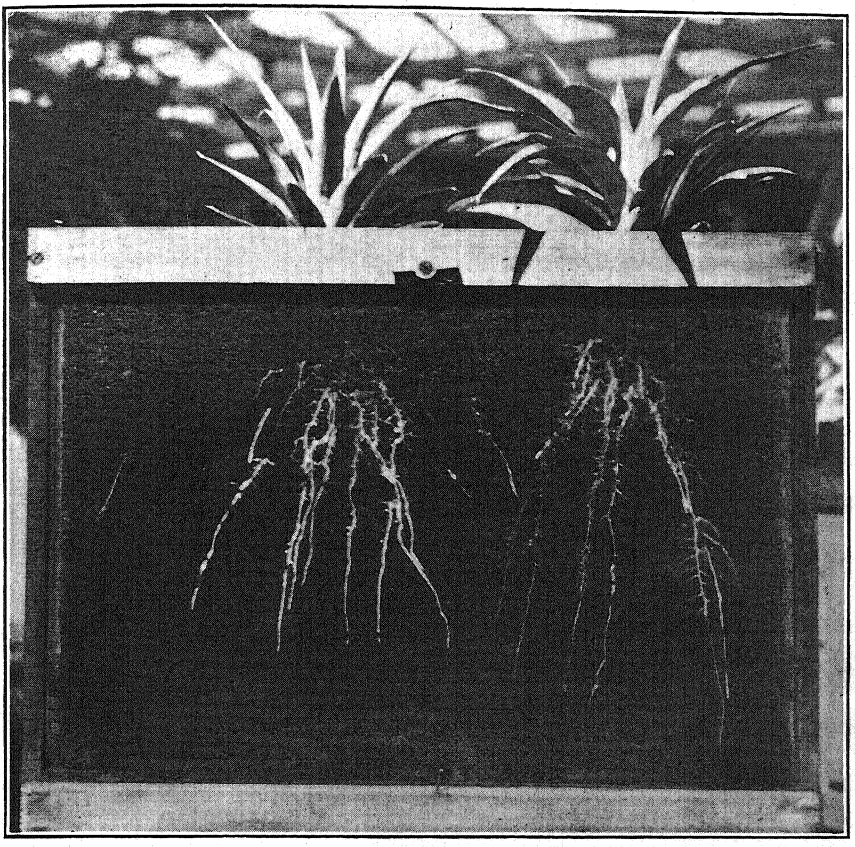


FIG. 5. Photograph of root observation box, showing the excellent availability of roots for observation, even with a microscope, and for manipulation. By simply unhooking and swinging back the upper cross-piece, and lifting the glass front, the roots are exposed without undue disturbance.

containing the Tylenchus, placed in tubs of sterilized soil which were later planted to cowpeas, resulted in extremely heavy infestation of this plant. Infested roots of cowpeas placed at the bases of young developing pineapple plants brought about heavy infestation of pineapple roots.

Many inoculations, reisolations of the Tylenchus, and reinoculations were carried through, so there can be no doubt but that the Tylenchus under consideration is the primary cause of the type of root lesions described above in pineapples and other plants.

DESCRIPTION OF THE CAUSAL NEMATODE

Tylenchus brachyurus, n. sp. The nematode causing this root lesion (Fig. 6) is a Tylenchus, very close in general appearance to *T. musicola*

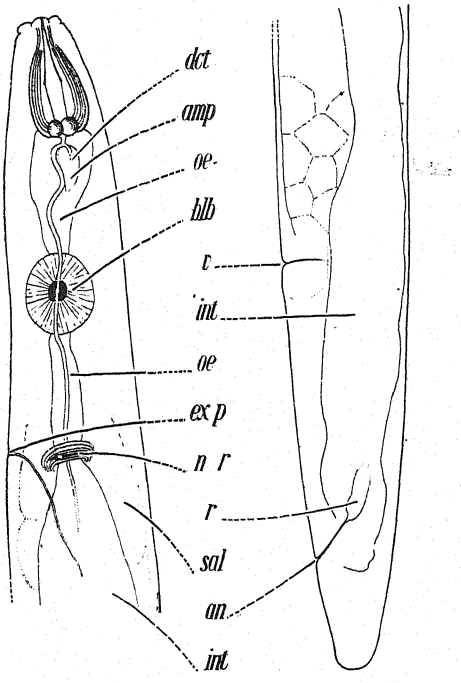


FIG. 6. Drawings of anterior and posterior ends of *T. brachyurus* n. sp. *dct*, outlet of salivary gland; *amp*, ampulla of salivary gland; *oe*, oesophagus; *blb*, median oesophageal bulb; *n r*, nerve ring; *ex p*, excretory pore; *sal*, salivary glands; *int*, intestine; *ov*, ovary; *v*, vulva; *an*, anus; *r*, rectum.

Cobb (3), to *T. pratensis* de Man (*T. penetrans* Cobb) (4, 9), to *T. coffeae* Zimmerman (10), and to *T. sacchari* Soltwedel (10). Contrary to the case with these species, however, only females have been found. Thousands of specimens have been examined in the search for males. The specimens studied were taken from lesions in all stages of development, and from surrounding soil as well. A search was made throughout the roots and soil of cultures resulting from inoculations made on growing roots in sterilized soil, in which many generations developed. If males occur they are exceedingly rare and not essential to reproduction.

Diagnosis: **Tylenchus brachyurus**, n. sp. A Tylenchus, close to *T. musicola* Cobb; differing from it by (1) thicker body throughout (thickness 23.2 μ); (2) consistently shorter proportional distance between the vulva and the terminus (14 per cent compared with 19 per cent) and shorter tail length (4 per cent compared with 7 per cent); (3) the noncuticularized, nonrefractive vulva and vagina wall; (4) absence of irregular extension at end of tail; (5) consistently larger eggs (eggs 80 x 38 μ); (6) absence of males. Measurements of adult female, average of eight specimens, are

included in the following graphic formula. Found principally in pineapple roots in the Hawaiian Islands.[3]

3.3	13.3	15.0	$^{43.5}86.0$	96.1	
					0.567 mm.
3.2	4.0	4.1	3.8	2.4	

The average of measurements of eight eggs is 0.080 mm. x 0.038 mm.

The outstanding differences between this species and *Tylenchus pratensis* are (1) longer and more massive spear in **T. brachyurus,** n. sp. (about 18 per cent greater), (2) about 33 per cent shorter proportional distance between the vulva and the terminus, giving the nematode its distinctly characteristic "short tailed" appearance, (3) the consistently larger eggs, and (4) the lack of males.

Compared to *T. coffeae* Zimm. and *T. sacchari* Soltw., the spear in our Tylenchus is about 20 per cent larger than in the one and 50 per cent larger than in the other; the body length and width are approximately the same in all; the tail is about the same as in *T. coffeae,* much shorter than in *sacchari;* the relative distance (in percentage of body length) from head to vulva is much greater, being 86 per cent in this Tylenchus, and in *T. coffeae* and *T. sacchari,* 80 and 81.2 per cent respectively. Besides these, other differences become manifest after closer study. Zimmerman pictures the ovary (10, figure 7) as extending forward to the front end of the intestine, which would make its length 75 per cent of the body length; in ours no specimen has been seen with an ovary length of more than 60 per cent, the average being considerably less than 50 per cent. The eggs of our species are larger than those of either of the others, as shown by the following comparison:

T. coffeae, 54 μ x 25 μ, *T. sacchari,* 100 μ x 25 μ, **Tylenchus brachyurus,** n. sp., 80 μ x 38 μ.

It is to be seen that **T. brachyurus,** n. sp., eggs are larger in both measurements than *T. coffeae* by about 50 per cent and that they are quite different in shape from those of *T. sacchari,* the latter being long and slender (length to breadth ratio being 4 to 1, while in **T. brachyurus,** n. sp., it is approximately 2½ to 1).

A very simple character by which one species of nematode may frequently be distinguished from another, both being well known to the observer, is the position the nematode assumes when at rest, or when dead,

[3] Differences between this species and *Tylenchus musicola* Cobb are even more clearly established by the detailed description of the latter by Goodey, in a paper received after this manuscript was submitted for publication. (Goodey, T. Observations on *T. musicola* Cobb, 1919, from diseased banana roots. Jour. Helminthology 6: 193–198. 1928.) Attention is called particularly to the differences in the tail region and to the relative position of the vulva.

as viewed under the low powers of a microscope. One Tylenchus encountered frequently in soils from pineapple fields, for instance, takes a very definite spiral position (like a watch spring) when at rest. **Tylenchus brachyurus,** n. sp., straightens itself out completely. In fact, even when active, it rarely assumes the spiral position. It is more apt to assume

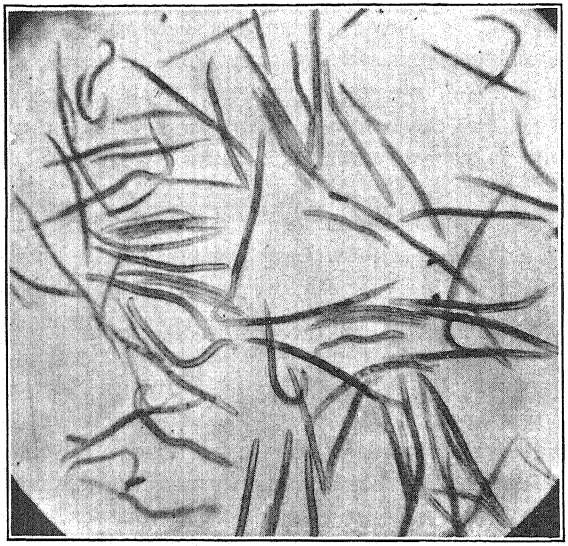


FIG. 7. Miscellaneous assortment of *T. brachyurus* eggs, larvae, and adults. When at rest, this nematode is usually straightened out, or only slightly bowed. When active, it is sluggish in its movements, and bends in the body are usually more or less angular—never spiral. Magnification about 30 diameters.

irregular angles at different points in the body. Figure 7 illustrates a miscellaneous assortment of **T. brachyurus,** n. sp., eggs, larvae, and adults, in normally quiescent and active positions, just as taken from a pineapple-root lesion.

A detailed description follows, including further characteristics, some of which would seem to indicate specific differences from any other known Tylenchus. The nematode would belong to Micoletzky's (7) subgenus Chitinotylenchus. There are six distinct lips. Thickened divisions between the lips are attached to the elongated guiding ring near the front end of the spear. This makes a very distinct cuticularized framework which, including the fore part of the spear, is sloughed off with the final moult, as is the case with *Tylenchus pratensis* and others.

There is no chitinization in the region of the vulva. The "lips" of the vulva protrude only slightly. A distinct rectum slightly anterior to the anus was observed in some specimens.

The lateral fields show four distinct longitudinal striae. Lateral wings, if they occur, are not pronounced. There are distinctly fewer annules in the lateral fields than on the dorsal and ventral fields. The posterior end of the left field and its relation to the tail are shown in figure 8, *a*.

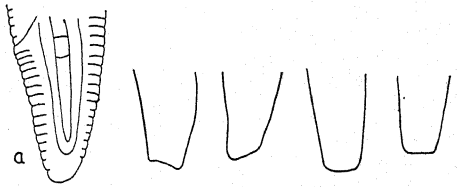


FIG. 8. Drawings of tail ends of *T. brachyurus*. The cuticular rings are shown in *a*; the other four show variations in the outline of the tail, all alike being left lateral views.

The tail is short, varying from 4 per cent to 5 per cent of the length of the body. It varies considerably in outline with different individuals. In figure 8, every drawing shows the left lateral view. There are usually about sixteen rings posterior to the anus, but in some unusually large specimens as many as twenty-two have been counted. There are between three hundred and four hundred rings in the entire body length.

Amphids are present, the sensillae extending back of the spear about half its length. The duct from the anterior salivary gland empties into the lumen of the oesophagus on its dorsal side a short distance back of the spear bulbs. In some specimens, under best conditions for observation, a suggestion of glandular tissue, set off from surrounding parts by its granular appearance, was seen just back of the spear, and extending backward to an undetermined extent. While all details could not be determined, it would appear to correspond with the "ampulla" described by Cobb (2) for *Tylenchus similis*. It is shown in figure 6. Muscles operating the spear are distinct. The fore part of the oesophagus is distinct, as shown by figure 6. It is wider in the anterior half, being about half the body width in this region, then narrows down posteriorly, to a relatively narrow cylindrical tube one-fifth the body width connected with the median oesophagus bulb, with a slight but distinct constriction. The spherical to ellipsoid median oesophageal bulb is typical of the genus. The rear portion of the oesophagus is less distinct, but in some specimens is visible as a somewhat narrow cylindrical tube, widening out at the posterior end, into the intestine, with no constriction. A nerve ring is visible just in front of the beginning of the posterior enlargement. The salivary glands may be seen obscurely, extending longitudinally posterior to the nerve ring. The excretory pore, on the ventral surface, is very distinct and has a

plainly visible long duct leading undoubtedly from a rennette gland somewhere posteriorly, beside the intestine, though its position was not determined. The relative position of the excretory pore with regard to the cardiac region is very irregular, being anterior, opposite, or posterior to it, in different specimens. A single anterior ovary varies in length between 30 per cent and 60 per cent of the length of the nematode, and is not reflexed. As a rule only one egg, and that unsegmented, has been found in the uterus. As many as four in a line, in different stages of development were found in one individual. Single eggs, segmented, have been observed, as have two together.

Some of the internal characters are difficult to determine in living specimens, owing to the abundance of fatty substance. By selection of specimens which appear clear under the dissecting binocular, the interior details can be determined more readily than in specimens not so selected. Excellent material for study of internal details was secured with specimens killed within the host tissues with Zenker's killing fluid, sectioned 6 and 8 μ thick, and stained with phosphotungstic acid haemotoxylin. Since most of the nematodes lie outstretched longitudinally in the root tissues (see next section), it is often possible to secure complete longitudinal sections.

Such sections showed to particularly good advantage the outlines of the oesophagus, including attachment to the intestine; also the nerve ring, and to some extent the glandular tissues. Nuclei were not deeply stained, so other details could be studied the more readily. Interestingly, this stain brought out in deep purple (or lighter, with lighter stained material) the muscle fibrils. These were prominent particularly in the muscles surrounding the spear, and this gave the head region an unusual appearance, partly, too, because the spear itself was rendered all but invisible. The muscle fibrils of the body-wall muscles were made to stand out prominently. These "strands," arranged longitudinally, and parallel and close to the body wall, gave the animal a distinct longitudinally striated appearance probably the "longitudinal muscular bundles" mentioned by Bastian (1, p. 80). In transverse mid-sections of an adult female nematode, ovary, intestine, and lateral chords are distinct, and muscle fibrils appear as black dots in dorsal and ventral body walls.

RELATION OF THE NEMATODE TO THE HOST TISSUES

As a rule, only the parenchyma cells of the cortex of a root are invaded by the nematode. The stele, after it has been differentiated in the region of branching, back of the region of elongation in a root, escapes penetration by the organism, at least for a long time. Consequently, a root may survive and grow for some distance even though the cortex surrounding it

in places may be completely invaded and killed. The organism seems to be limited by lignified cell walls. Thus even a slight degree of hardening of the epidermal cells prevents primary penetration on the part of nematodes from the soil, and also prevents the spread of the lesion itself, superficially. Lesions spread almost entirely subepidermally, after primary penetration of the young root has occurred.

The nematodes are, at best, sluggish in their activities. Fast growing roots will usually grow away from individuals that may have penetrated at or near the growing tip of the root. This accounts for the fact that most of the lesions occur on the sides of a root, with the growing tip apparently untouched. It is only in a case of heavy infestation of the soil or where the root has in some way been retarded in its forward progress that serious invasion takes place in the root tip. This constitutes the principal exception to the rule of invasion of the cortex only; and a serious exception it is for the welfare of the plant. When the nematodes become established in sufficient numbers in the meristematic tissue of the root tip, the root is quickly killed. Plate XV, *B*, shows a root tip thus invaded. It is to be noted too that when a root is thus severely attacked the young branches arising in the region of branching, an inch or two back of the tip, are often attacked and prevented from development. Such branch roots with the discolored portions invaded by nematodes, are shown in Plate XV, *A* and *B*, and in text figure 2, in which in *a*, most of the important lateral "feeding" roots are destroyed; whereas, in *b*, a normal root, they are fully functioning. Cases have been observed in which primary invasion was directly through the root cap.

As was stated before, the stele usually escapes penetration and the root continues to grow in spite of the invasion of the cortex. Initial infections in the upper part of young roots may continue to develop in the cortical cells, however, until the cells are entirely destroyed in the entire circumference of the root, and for several inches of its length. Such heavy infections may eventually reach a stage when, either directly or by means of toxins or secondary organisms, the vessels of the vascular system are killed. This appears to happen rather suddenly, for many roots on a given plant have been observed in different stages of infection, some of them with apparently dead tissues throughout the root in the region of the large lesions described, and all still showed the root tips to be healthy or barely wilted. Such roots rapidly deteriorate and eventually decay. Figure 1 shows such roots, healthy in the lower portions, but completely girdled by Tylenchus lesions in the regions indicated. Much of the death of long well-developed pineapple roots can be attributed to this cause.

Most of the nematodes abandon infested regions, shortly after the tissues have been killed. A great many roots have been examined, in which the

typical symptoms in the cortex indicated that heavy infestation had once been present, but which showed a very small present population. The nematodes, larvae and adults alike, migrate into the soil and seek new young roots. In the pineapple plant, root development is to a certain extent by spurts, depending upon the degree to which conditions favor growth. The new roots, growing down through the soil occupied by a first lot of roots badly damaged by Tylenchus, are often attacked in an early stage, with subsequent killing in the manner described in the previous paragraph.

Excellent low-power observations on the location and arrangement of the nematodes within the host tissues were obtained by a process which I developed as a modification of Arzberger's method of studying mycorrhizal fungi, while working at Madison, Wisconsin, from 1921 to 1923, and which I have modified somewhat since. Steiner has mentioned this method of study of nematodes within roots, or something similar to it (8), and has used it for observations and illustrations (9). My method consists of killing the tissues with hot (approximately 50° C.) Flemming's medium or strong killing fluid, leaving for a half hour or so only, transferring to chrom acetic fixing fluid for 12 to 24 hours, washing, dehydrating, and clearing in clove or some other oil. The osmic acid of Flemming's fluid stains the nematodes without staining the plant tissues, so that in the cleared specimen the nematodes stand out clearly. Figure 10, *a*, is a photograph of such a view of **T. brachyurus**, n. sp., in a pineapple root, and figure 9, b, of those within lesions in a cowpea root. Adults, larvae, and eggs may sometimes be seen in great numbers in the tissues. They usually are to be found at the advancing ends of the lesions rather than in the center, where the tissues have disintegrated. The nematodes work their way through the tissues, feeding as they progress. They are not confined to the intercellular spaces, but break readily through the cell walls. A row of two or three to half a dozen or more eggs, evidently deposited by a single adult female, may often be seen just back of her in the damaged tissues.

Materials for the study of histological and cytological details, in young lesions, were killed with hot (50 to 55° C.) killing fluids of different kinds, washed, dehydrated, and infiltrated with paraffin according to the usual methods. Young lesions, near the tips, could be very satisfactorily processed for sectioning. Older lesions, farther up on the roots, contained so much silicified tissue that they could not be cut without first treating with hydrofluoric acid for ten days or two weeks, prior to dehydration. The histological work reported herein was done by Dr. R. C. Whitman, of the University of Colorado, who is spending his sabbatical leave at the Pineapple Experiment Station.

A careful study of sections 6 and 8 μ thick verifies conclusions from macroscopic examinations that lesions spread within the cortex subepider-

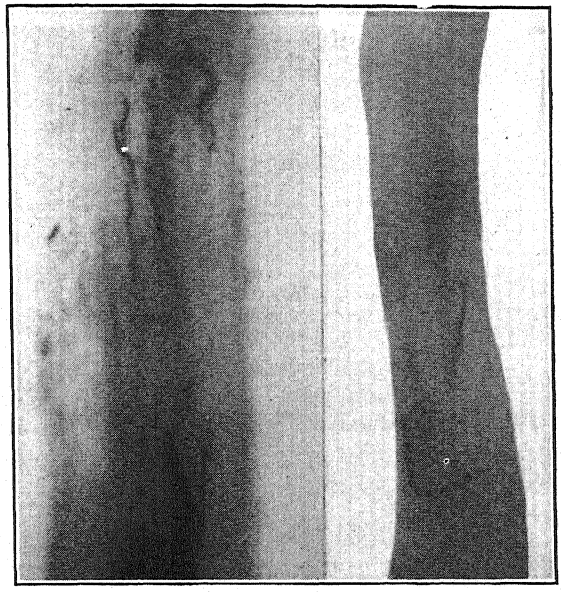


FIG. 9. Photographs of nematodes within roots; *a*, pineapple, and *b*, cowpea. The roots were first killed with a killing fluid containing osmic acid (warm for instantaneous killing), fixed with chrom acetic fluid, washed, dehydrated, and cleared in clove oil. This is an excellent way to demonstrate nematodes in plant tissues.

mally and mostly in a longitudinal direction. The nematodes enter the roots through the epidermal cells, sometimes through root hairs. They force their way through the cells, destroying them as they go. Apparently, they are limited not at all by the cell walls of the cortical parenchyma. As a rule, they at first move along longitudinally in the root in the outer two or three layers of cells. Cross sections almost invariably show clean-cut transverse sections of the nematodes in this region. They then turn inward and move through the tissues either singly or in groups of several, causing quick and complete death of the cells they penetrate and disintegration of the cell contents. A distinct discoloration of the tissues follows quickly after penetration by the nematodes. To what extent secretions play a rôle in the death of the cells is not clear. It is certain, however, that uninjured cells with the nuclei intact have been seen immediately adjoining the body of the nematode. (See Fig. 10, *a* and *b*.) Considerable regions in a root are often killed by nematodes and then are completely abandoned by them. I am not always able to decide with certainty whether certain cells, obviously dead, have been killed by direct invasion, possibly penetration of the spear alone (the walls being apparently intact), or whether they have been killed by toxic secretions from the nematodes, or by some other means. Possibly

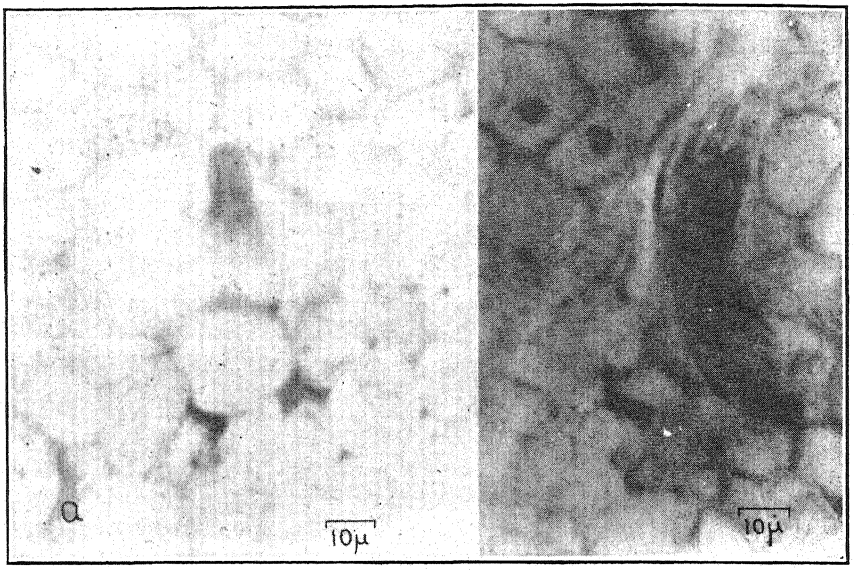


FIG. 10. Photomicrographs showing *T. brachyurus* within the cortical tissues of pineapple roots. Note the uninjured host nuclei in cells immediately adjoining the body of the nematode. In other cells, already traversed by the nematodes, the cells are killed, and cell contents disintegrated.

death of such cells may sometimes be physiological, due to the complete interference with normal nutrition brought about by the killing of a considerable region of adjacent tissue.

The older lesions are frequently accompanied by invasions by secondary organisms. Bacteria have often been seen which had advanced intercellularly to the very limits of invasion by the nematodes. Fungi also have been seen in sections of roots attacked by the Tylenchus, obviously as secondary invaders in nematode invaded tissues; sometimes they appear to have advanced from lesions into otherwise uninjured tissues. A species of Cephalobus, a nematode of doubtful parasitic habits, has been seen within Tylenchus lesions; it is invariably surrounded and preceded by bacteria. In younger lesions, many advancing edges have been studied that appear to be completely sterile in so far as organisms other than the Tylenchus are concerned.

SOME OBSERVATIONS ON ENVIRONMENTAL RELATIONS

Detailed work on environmental relations of **Tylenchus brachyurus**, n. sp., has not been done. However, miscellaneous observations, some of which are significant, have been recorded from time to time. The impres-

sion has been gained, from laboratory observations, that the Tylenchus is more resistant to adverse conditions, particularly to drying, than is Heterodera. Mounts left standing on slides, or suspensions in dishes left to complete dryness are more apt to show recovery to activity of some individuals than is Heterodera. Living specimens can frequently be recovered from field roots that appear to be dry.

Accurate observations were made in conjunction with studies on the drying of soils containing primarily *Heterodera radicicola* (6) but having a heavy Tylenchus population as well. In this test, a large volume of soil was uniformly heavily infested with both Heterodera and Tylenchus by mixing with it the roots of sixty pineapple plants heavily infested with both nematodes. The soil was divided among several large wooden tubs filled to a depth of ten inches. Tubs were subjected to several different conditions, including in one lot, continuous drying with complete stirring once a week, and in another lot the maintenance of the soil under good conditions for growth, in so far as watering was concerned. Four months of complete drying eliminated the last trace of living Heterodera, as evidenced by cowpeas as an indicator crop. The same indicator crop showed, however, that the Tylenchus survived to a surprising degree. Some of the plants showed heavy infestation; in fact, figure 4 is taken from one of the plants of this lot. The Tylenchus was shown to be capable, in an inactive state, of surviving over a considerable period, and of quickly resuming activity and capacity for infecting plant roots on the restoration of favorable conditions. One of the measures recommended for the control of the root-knot nematode, namely continuous dry fallow with frequent stirring of the soil for several months during a dry period, will not, therefore, be effective in eliminating the Tylenchus. An encouraging feature developed, however, in the continuously moist series of tubs. Here it was determined that after four months a very much lower Tylenchus population was left; less than 1 per cent of that in original plantings. The conditions being favorable for the continuous activity of the nematode, in its search for food it depleted its store of reserves, and starved.

Observations on pineapple-field soils, including careful nematode determinations through a series of fallow treatments, have shown definite reduction of Tylenchus as well as of Heterodera (6). On the basis of these various observations, it is certain that the Tylenchus population of pineapple-field soils may be greatly reduced in the inter-crop period by a period of clean fallow during moist weather. Possibly the growing of immune crops during this period will have the same effect. Such procedure can be made to fit very well with the root-knot-nematode reduction program. Field tests are under way to get additional definite information on this point.

SUMMARY

One factor contributing to root failure in pineapples in Hawaii is a root lesion produced by a species of the nematode genus Tylenchus. Lesions on young roots are irregular, longitudinal, argus brown spots, from one to several millimeters long, extending through cortical parenchyma cells but not into the stele. The nematodes multiply and migrate in the cortex, destroying the cells, until finally the root is killed. Branch feeding roots are often greatly reduced in number. The nematodes are sluggish, and lightly infested roots grow away from them and continue to function. When soil infestation is sufficiently heavy so that numbers of nematodes invade root tips, severe injury results and further growth of the root is prevented. Injury to the root tissues appears to be purely mechanical. Histological studies show root cells adjacent to the invading nematode with nuclei intact. Nematode root knot checks forward growth, making conditions ideal for penetration and multiplication of the Tylenchus, which then rapidly breaks down the tissues. Presence of the Tylenchus in a gall is always indicated by a brownish color. The two nematodes together make a destructive combination. Other plants subject to attack are tomato (slightly), soybean, cowpea (very heavily) and many weeds. In contrast with *Heterodera* (*Caconema*) *radicicola* the Tylenchus is resistant to drying. Its numbers are greatly reduced, however, in moist soil fallow, probably by starvation. The nematode is described as **Tylenchus brachyurus**, n. sp.

EXPERIMENT STATION, ASSOCIATION OF
HAWAIIAN PINEAPPLE CANNERS.

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EXPLANATION OF PLATE XV

Tylenchus brachyurus n. sp. in pineapple and cowpea roots.

A to G. Typical lesions in pineapple roots.

A and B. Severe injury in main root and laterals. Root knot symptoms, produced by *Heterodera radicicola,* are evident as well.

C and D. Surface view and longitudinal section, respectively, of a root tip invaded by both the Tylenchus and the Heterodera. The region invaded by the Tylenchus is evidenced by the dark color.

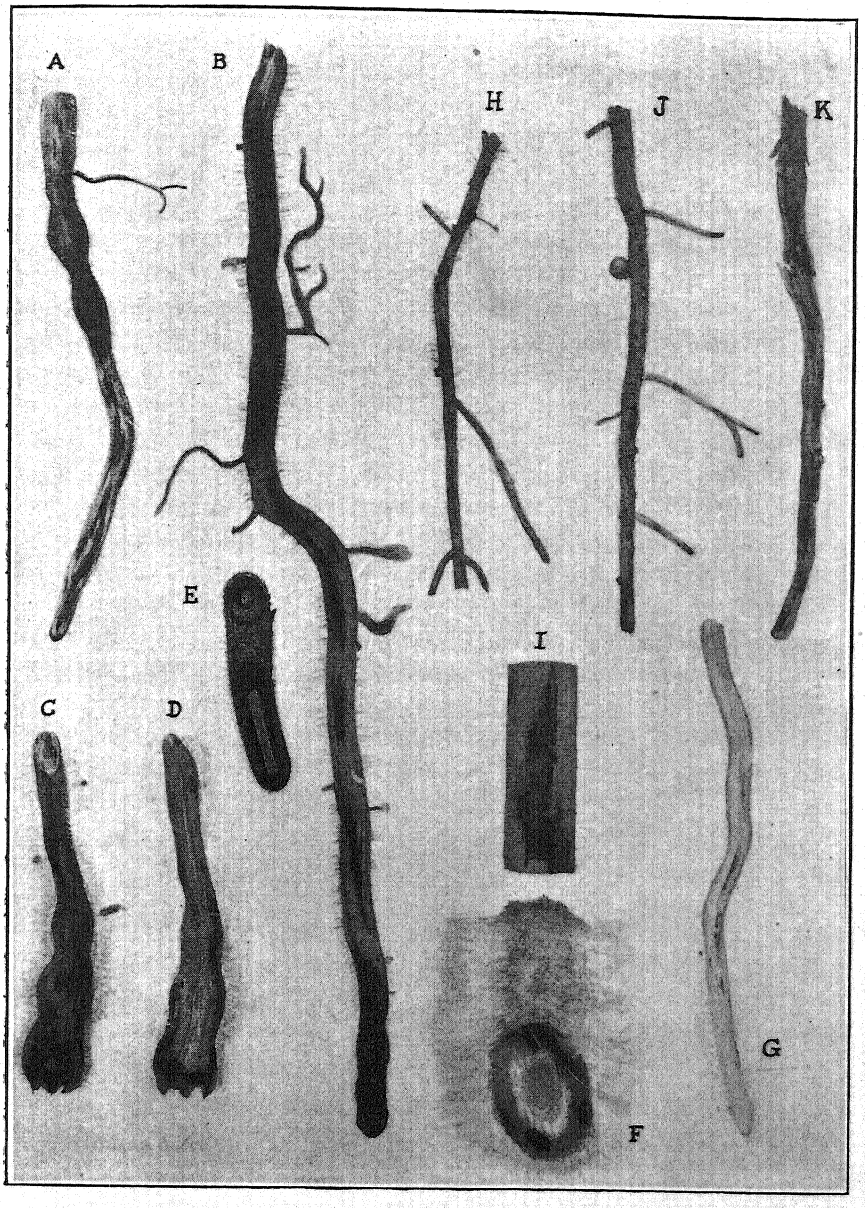
E. A portion of an older root that has been killed by the advance of the Tylenchus within the cortex. Note that the lesion has spread subepidermally until the entire cortex has become brown.

F. A somewhat younger root than that shown under E, the root hairs being still intact. The depth of penetration of a lesion is here evident.

G. Very young lesions on a pineapple root.

H to K. Lesions in a cowpea root. The one in I is the same as the lower one in H, magnified about five diamaters. In K the tissues are beginning to disintegrate, consequent to the invasion of secondary organisms.

The lesions are, in color, very close to the "argus brown" of Ridgeway.


A
B
H
J
K
E
I
C
D
G
F

SULPHUR DUSTING FOR THE PREVENTION OF STEM RUST OF WHEAT[1]

E. B. LAMBERT AND E. C. STAKMAN

INTRODUCTION

For many years it was assumed that *Puccinia graminis* Pers., the fungus causing stem rust of small grains, could not be controlled either by spraying or dusting. Kellerman (6), Pammel (10), and Galloway (4) applied a number of fungicides to growing plants to ascertain whether the development of rust could be prevented. They used both sprays and dusts, including flowers of sulphur, but the results were not at all encouraging. The dusts were ineffective and spraying large acreages of small grains with a liquid fungicide seemed to be impracticable and probably would have proved to be so. With the development of improved fungicidal dusts, however, attention again was called to the possibility of controlling cereal rusts by dusting. Some preliminary tests[2] were made under the direction of the junior author at University Farm, St. Paul, Minn., in 1923. Sulphur dust was applied to wheat plants, but there appeared to be no beneficial effect, probably because the dust was not fine enough or was not applied at the proper time.

In 1924 there was very little stem rust in the spring wheat area, and no experiments were made. In 1925, however, trials were made on a more adequate scale than in 1923. The results were unusually satisfactory, and experiments therefore were again made in 1926. In the meantime, however, Kightlinger (7) published the results of experiments made at Cornell, and to him must go the credit of having been the first to demonstrate the possibility of controlling cereal rusts by dusting growing plants with sulphur.

In 1926, Kightlinger and Whetzel (8), Bailey and Greaney (1), and Lambert and Stakman (9) published results of dusting trials in New York, Manitoba, Minnesota, and North Dakota, respectively. Since that time Bailey and Greaney (3) and Greaney (5) have published detailed summaries of their subsequent results.

[1] Cooperative investigations between the Minnesota Agricultural Experiment Station and the Office of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Published with the approval of the Director as Journal Series Paper No. 838, University of Minnesota, University Farm, Minn.

[2] These experiments were made at the suggestion of Prof. H. H. Whetzel, who stated that some of the newer sulphur dusts were so superior to ordinary sulphur that they merited trial as preventives of rust.

After 1926, the Minnesota experiments were continued by W. C. Broadfoot, who made much more extensive experiments in 1927 and 1928. The results of Broadfoot's experiments are being published separately. It seems worth while to publish also the results obtained by the writers in 1925 and 1926, as it is essential to have data for a number of years in order to determine the practical value of dusting.

EXPERIMENTS IN 1925

In 1925, Marquis wheat was dusted with precipitated sulphur at Excelsior, St. Paul, Morris, and Crookston, Minn.; and at Glasston and Tolley, N. D. At Excelsior the experiments were made in a low field. The plots were marked off by stakes in the lowest part of the field, where the likelihood of severe rust attack was greatest. The dust was applied with a hand duster, at a rate of approximately 150 pounds per acre, which was sufficient to cover the plants thoroughly. Treated plots and untreated check plots alternated.

Primary infection by stem rust was first observed in this field on June 17. At the time of first dusting, on June 25, stem-rust spots due to secondary infection were about 1 foot in diameter and occurred, on the average, at 6-foot intervals throughout the field. The wheat plants were flowering on June 25, were in the milk stage on July 2, and were in the hard dough stage on July 17.

The results of the treatments are given in table 1, from which it is evi-

TABLE 1.—*Results of dusting Marquis wheat at Excelsior, Minn., with precipitated sulphur at the rate of 150 pounds per acre, in 1925*

Plot No.	Plots treated (×) on		Stem-rust severity, in the rusted plots, on July 17
	June 25	July 2	
1	×	×	Trace to 5 per cent on peduncles; 40 per cent on sheaths
Check	–	–	80 per cent
2	×	–	80 per cent; plants slightly greener than checks
Check	–	–	80 per cent
3	×	×	Trace to 5 per cent on peduncles; 40 per cent on sheaths
Check	–	–	80 per cent
4	×	×	Trace to 10 per cent on peduncles; 40 per cent on sheaths
Check	–	–	80 per cent
5	×	–	80 per cent; plants slightly greener than checks
Check	–	–	80 per cent
6	–	×	Trace to 10 per cent on peduncles; 40 per cent on sheaths
Check	–	–	80 per cent

dent that one application of dust on July 2, when the wheat plants were in the early-milk stage of development, reduced the rust to a remarkable degree. Two applications, the first on June 25, when the wheat was heading, and the second on July 2, resulted in no greater reduction of rust than did the single application on July 2.

It is clear that one application of dust may control rust, but only if made at the proper time, which, for this field, was about July 2. The first application, made on June 25, seemed to be of little or no value. Apparently it was made too early, and either too much of the dust had been washed off by the time large numbers of rust spores reached the plants, or it was no longer chemically effective.

The field was harvested before material was obtained for determination of yields, but it was perfectly apparent, even on casual observation, that the bundles of dusted wheat were far heavier than those of the non-dusted.

At St. Paul the experiment was more extensive but there was less rust. These trials were made in two fields. The first, field number 1, was adjacent to an artificially inoculated rust nursery. In all probability this accounts for the earliness and the irregularity of the infection. A trace of rust was already present in all of the plots at the time the wheat was flowering. By June 16 all culms were infected. The stages of development of the wheat on the dates the dust was applied are as follows: June 20, flowering; June 25, flowering to milk; July 3, milk; July 7, milk to soft dough, and July 13, soft dough. On July 16, when the final rust notes were taken, the wheat was in the hard dough stage. It was approximately mature by July 20. Field 2 was sown later than field 1 and was located about a half mile from the rust nursery. Traces of rust did not appear in this field until about July 16. At that time the wheat varied from the flowering to the milk stage. It was ripe when the final rust notes were taken. The results obtained in these two fields are presented in tables 2 and 3.

From a study of the data in these two tables, it is apparent that stem rust was controlled only when the dust was applied at a certain stage in the development of the host and its parasite. On one plot, plot 11 (table 2), in which the dusting was discontinued too early, stem rust appeared just before the straw hardened, even though five applications were made. On the other hand, in certain other plots (table 3), the dust apparently was applied too late to be effective, although very little rust was visible when the dust was applied.

At Crookston and Morris the plots were dusted too late. Precipitated sulphur dust was applied to the Crookston plots at five-day intervals at the rate of 150 pounds per acre. The first application of dust was made when the wheat was in the early milk stage, July 16. At that time there was no

TABLE 2.—*Results of dusting Marquis wheat in Field 1, University Farm, St. Paul, Minn., with precipitated sulphur at the rate of 50 pounds per acre, in 1925*

Plot number	Date of dust application					Percentage of stem-rust severity	
	June		July				
	20	25	3	7	13	July 16	July 20
1	×	×	×	×	×	5	5
2	–	×	×	×	×	5	5
Check	–	–	–	–	–	5	5
3	×	×	×	×	×	10	5
4	–	×	×	×	–	5	5
Check	–	–	–	–	–	5	5
5	–	×	×	–	–	5	5
6	×	–	×	–	–	5	5
Check	–	–	–	–	–	5	5
7	–	×	–	×	–	10	10
8	–	×	–	–	–	15	15
9	–	–	–	–	–	15	20
Check	–	–	–	–	–	15	20
10	–	–	–	×	–	25	30
Check	–	–	–	–	–	60	65
11	×	×	×	×	×	15	65
Check (alley)	–	–	–	–	–	60	65
Check	–	–	–	–	–	30	40
12	–	×	×	×	×	10	40
13	×	×	×	×	–	5	40
Check	–	–	–	–	–	5	30
14	–	×	×	×	–	5	10
15	–	×	×	–	–	5	5
Check	–	–	–	–	–	5	5
16	×	–	×	–	–	5	5
17	–	×	–	×	–	5	5
Check	–	–	–	–	–	5	5
18	–	×	–	–	–	5	5
19	–	–	×	–	–	5	5
Check	–	–	–	–	–	5	5
20	–	–	–	×	–	5	5

rust on the wheat peduncles, but it had appeared on them in all plots by July 19. On July 26 there was 85 per cent to 95 per cent stem-rust severity on all culms in both dusted and non-dusted plots. Since the minimum incubation period is about five days, it was apparent that the rust had already penetrated the wheat plants before the dust was applied. The results obtained at Morris were essentially the same as those at Crookston.

TABLE 3.—*Results of dusting Marquis wheat in Field 2, University Farm, St. Paul, Minn., with precipitated sulphur at the rate of 50 pounds per acre, in 1925*

Plot	Date of dust application						Per cent of stem-rust severity
	July				August		
	16	21	25	30	3	10	August 11
1	×	×	×	×	×	×	trace
2	×	×	×	×	–	–	trace
Check	–	–	–	–	–	–	45
3	×	×	×	–	–	–	5
4	×	×	–	–	–	–	15
Check	–	–	–	–	–	–	45
5	×	–	–	–	–	–	10
6	×	×	×	×	×	–	5
Check	–	–	–	–	–	–	25
7	–	×	×	×	×	×	50
Check	–	–	–	–	–	–	50
8	–	–	×	×	×	–	50
Check	–	–	–	–	–	–	60
9	–	–	–	×	×	–	50
Check	–	–	–	–	–	–	40
10	–	–	–	–	×	–	40
Check	–	–	–	–	–	–	50

Experiments also were made at Glasston and Tolley, North Dakota. The dust was applied at the rate of 50 pounds per acre. In different plots, 1, 2, 3, and 4 applications were made at 5-day intervals. In most cases the dusting was begun when the wheat was in the milk stage. Here, too, infection apparently had already occurred before the first applications were made. At harvest time there was no appreciable difference between the percentages of rust severity in the dusted plots and those in the checks except in a very late field at Tolley, in which dusting was begun at heading time. In this field two applications of dust reduced the percentage of rust severity from 75 per cent in the check plots to 15 per cent in the treated plots.

The results of these preliminary experiments indicate clearly that, under certain conditions, sulphur dust does control stem rust, even in the presence of a severe epidemic. However, the dust seemed to have little or no effect on the development of rust pustules after the pathogene had entered the host. Furthermore, it did not seem effective for more than a few days after it had been applied. Timeliness and frequency of applica-

tion were the critical factors. The best time to begin dusting appeared to be when the peduncles of the wheat plants first became fully exposed. Although one and two applications effectively controlled rust in a few cases, several applications made at 5-day intervals, the first when the wheat was heading, seemed to be necessary to insure rust control.

It was apparent that further experiments were needed to determine the relative effectiveness of different kinds of dusts and to devise the most economical program of applications for rust control. Therefore, the experiments were continued in 1926.

EXPERIMENTS IN 1926

In 1926 tests were made at St. Paul, Morris, and Crookston, Minnesota. At St. Paul two dusting programs were used, one similar to that used at Morris and Crookston, and the other in which experiments were made with five kinds of commercial sulphur dusts[3] applied at three rates: 50, 100, and 150 pounds per acre at each application. At Morris and Crookston[4] two kinds of dust were applied, Kolodust and Anchor Brand (flowers of sulphur), at the rate of 50 pounds per application per acre. All of the plots were replicated three times. The experiments were arranged to determine the most desirable brand of dust, the proper time to begin dusting, the smallest number of dustings that would control rust, the most satisfactory interval between dustings, and the effect of different rates of application.

The schedule followed in the experiments at Crookston is given in table 4. Similar dusting programs were followed at Morris and at St. Paul. But in these more southern latitudes the dusting was begun and discontinued 10 days earlier than at Crookston. The first application of dust was made approximately at flowering time. As indicated in table 4, 1, 2, 3, and 4 applications, respectively, were made during each of three different time periods in the development of the crop. Applications also were made at 3-, 5-, 7-, 9-, and 11-day intervals; and in one case the dust was applied to the soil only.

Some rust developed in the late plantings at St. Paul, but there was very little in the plots at Morris or in the early-sown plots at St. Paul. The best results were obtained at Crookston, as more rust developed there than

[3] Kolodust, sold by the Niagara Sprayer Co., Middleport, N. Y.; Anchor Brand dust, sold by the Stauffer Chemical Co., Houston, Texas; Sulphur Smoke, sold by the Nitrate Agencies Co., Bayonne, N. J.; Brooklyn Brand (No. 300–7), sold by Battele and Renwick, New York City; No. 300–7 sulphur dust, sold by National Sulphur Co., New York City.

[4] The work at Morris was done in cooperation with Mr. R. O. Bridgford; at Crookston in cooperation with Mr. R. S. Dunham.

TABLE 4.—*Dusting program at Crookston, Minn., in 1926*

Plot No.	Kind of dust	Number of dustings	July 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	"Kolo"	Check																														
2	"	1										×																				
3	"	1															×															
4	"	1																				×										
5	"	2										×					×															
6	"	2										×							×													
7	"	2										×									×											
8	"	Check																														
9	"	3					×					×					×															
10	"	3										×					×					×										
11	"	3															×					×				×						
12	"	4					×					×					×					×										
13	"	4										×					×					×				×						
14	"	4															×					×				×						×
15	"	Check																														
16	"	Continuous					×			×			×			×			×			×			×			×			×	
17	"	"					×					×					×					×				×						×
18	"	"					×							×							×						×				
19	"	"					×									×									×						
20	"	"					×											×										×			
21[1]	"	"					×			×			×			×			×			×			×			×			×	
22	"	Check																														
23	"	–			Before rain																											
24	"	–			After rain																											
25	"Anchor"	Continuous					×			×			×			×			×			×			×			×			×	
26	"	"					×					×					×					×					×					×
27	"	"					×							×							×						×				
28	"	"					×									×									×						
29	"	"					×											×										×			
30[1]	"	"					×			×			×			×			×			×			×			×			×	
31	"	–			Before rain																											
32	"	–			After rain																											
Precipitation in inches					.06			tr		.26	.28						.17				.17		.09	.04	.21							

[1] Dust applied on soil only.

at any of the other stations. The schedules of application followed at each station and the results obtained are given in tables 5 and 6.

There was too little rust in the plots at Morris and in the early-sown plots at St. Paul to permit definite conclusions. Yield tests were made but, owing to a severe drought in May, the stands were so irregular that in many cases there was a difference of 30 per cent between the yields of different replications.

The late field at St. Paul was sown on May 17. It was not injured so severely by the drought and became moderately infected with stem rust. The wheat headed July 10 and the first pustules of rust appeared July 21. There was rust on the peduncles August 2. All of the culms were infected in the check plots. The effect of dusting on the percentage of stem rust at harvest time is given in table 5.

A comparison of the amount of rust in plots 22, 25, 28, 32, and 35 indicates that "Kolodust" was the most effective of the dusts tested, with the possible exception of "Sulphur Smoke." The 50-pound applications of Kolodust seemed to control the rust as well as 150-pound applications, but there was not enough rust in the plots for a conclusive demonstration.

At Crookston there was enough rust in the check plots to determine clearly the effect of the different dustings. As the 1925 experiments had indicated that dusting would not be effective if begun after the wheat had passed the flowering stage, most of the plots at Crookston, in 1926, were dusted first at blossoming time. The wheat was sown on April 16; it headed on July 8; blossomed on July 10; and was ripe on August 10. The first pustule of rust appeared on July 11. The effect of dusting on the amount of rust at harvest time and on the yield is summarized in table 6. The amount of stem rust at harvest in plots 11 and 14 indicates that equally favorable results would have been obtained had the first application been made on July 15, because the rust developed about a week later at Crookston in 1926 than in 1925.

Evidently no fixed rule can be followed regarding the best time to begin dusting. The experimenter is distracted by the desire on the one hand to dust early and insure rust control, and on the other hand to dust late and save dust. Seasonal fluctuations and isophane differences make it impossible to recommend a definite date which would be equally applicable in all seasons and in all parts of the spring-wheat area. Theoretically, the most satisfactory time to begin dusting in a given region is when stem rust first can be found on wheat. But this criterion can not be used in practice unless the rust development is watched by trained observers. The most promising solution of the problem is to recommend dusting at a certain growth stage of the wheat plant. In the spring-wheat area stem rust, as a

TABLE 5.—*Results of dusting Marquis wheat with five different brands of sulphur in late-sown plots, at St. Paul, in 1926*

Plot No.	Kind and amount of dust per acre	Date dusted	Per cent stem rust severity at harvest time		
			Plots		
			A	B	C
1		Check	5	10	5
2		7/17	5	8	5
3	Kolodust	7/22	5	5	tr
4	50 pounds	7/27	7	8	5
5		8/1	8	5	3
6		7/17, 22	tr	2	tr
7		7/17, 24	tr	2	5
8		7/17, 22, 27	tr	tr	tr
9		7/17, 24, 31	5	3	3
10		7/21, 24, 27, 29, 8/4	4	5	4
11		Check	7	10	15
12	Kolodust	7/17	5	10	6
13		7/22	5	15	5
14	150 pounds	7/27	5	2	6
15		8/1	15	10	2
16		7/17, 22	3	5	1
17		7/17, 24	10	10	2
18		7/17, 22, 27	5	3	tr
19		7/17/24, 31	5	3	tr
20		After rains	20	2	3
21		Check	8	10	8
22	Kolodust	7/12, 17, 22, 27, 8/1, 7	tr	tr	tr
23	100 pounds	7/12, 19, 26, 8/2	5	5	4
24		7/12, 22, 8/1	tr	5	tr
25	Anchor	As No. 22	8	8	8
26	100 pounds	As No. 23	8	8	8
27		As No. 24	8	10	4
28	Smoke	As No. 22	2	3	5
29	100 pounds	As No. 23	3	5	8
30		As No. 24	2	3	10
31		Check	5	8	10
32	National Sulphur	As No. 22	2	3	10
33	Co. 100 pounds	As No. 23	8	8	5
34		As No. 24	5	6	5
35	Battele & Renwick	As No. 22	3	2	5
36	100 pounds	As No. 23	4	6	8
37		As No. 24	8	4	3

rule, does not become abundant until after blossoming time. In all of our experiments entirely satisfactory results were obtained by applying the first dust at this time. Therefore, until further experiments have been made, blossoming time is recommended as the best time to begin dusting.

The most satisfactory interval between dustings seems to be from 5 to 7 days. A comparison of the results obtained in plots 16, 17, and 18 (Table 6) shows that applications at 5-day and 7-day intervals will control rust as effectively as those at 4-day intervals. In all of the replications in plots

TABLE 6.—*Results of dusting Marquis wheat with Kolodust and flowers of sulphur at the rate of 50 pounds per acre at Crookston, Minn., in 1926*

Plot No.	Date dusted in July	Per cent rust severity						Yields in grams from Plots A		Grade
		July 29			August 7					
		Plots			Plots			2 sq. yd.	200 seeds	
		A	B	C	A	B	C			
1	Check	15	8	–	75	65	–	297	49.75	3 DN
2	10	5	tr	–	70	65	–	311	55.60	1 DN
3	15	5	3	–	70	55	–	298	59.40	1 DN
4	20	10	6	–	70	45	–	261	55.05	1 DN
5	10, 15	tr	tr	–	55	35	30	304	61.15	1 HS
6	10, 17	tr	4	1	35	35	7	326	63.65	1 HS
7	10, 19	tr	2	4	40	45	15	373	65.15	1 HS
8	Check	5	5	12	70	50	45	315	56.70	2 DN
9	5, 10, 15	tr	tr	4	45	20	30	339	64.25	1 HS
10	10, 15, 20	tr	tr	tr	5	2	10	274	59.30	1 HS
11	15, 20, 25	2	tr	3	5	2	2	331	64.75	1 HS
12	5, 10, 15, 20	tr	tr	tr	5	3	2	289	62.35	1 HS
13	10, 15, 20, 25	tr	tr	tr	tr	2	2	410	65.10	1 HS
14	15, 20, 25, 30	tr	tr	1	2	2	2	302	64.55	1 HS
15	Check	5	1	6	25	5	35	326	60.70	1 DN
16	5, 8, 11, 14, 17, 20, 23, 26, 29, 8/2	tr	tr	tr	tr	tr	3	339	66.80	1 HS
17	5, 10, 15, 20, 25, 30	tr	4	tr	tr	tr	3	373	69.15	1 DN
18	5, 12, 19, 26	tr	1	tr	tr	tr	tr	338	61.50	1 N
19	5, 14, 23, 30	tr	1	tr	1	2	3	371	65.30	1 N
20	5, 16, 27	tr	5	6	1	5	5	438	64.70	1 RS
21	As No. 16 but on soil only	3	8	5	3	40	45	409	65.75	1 RS
22	Check	5	10	14	30	65	65	396	61.10	1 RS
23	—	8	5	7	20	60	65	334	63.60	1 RS
24	After rain	tr	1	tr	2	2	3	341	64.00	1 RS
25	As No. 16	10	1	2	5	tr	tr	373	61.05	1 DN
26	5, 10, 15, 20, 25, 30	5	1	3	10	3	10	382	61.55	1 HS
27	5, 12, 19, 26	5	6	4	15	20	10	403	57.50	1 HS
28	5, 14, 23	5	4	5	30	20	35	389	56.85	1 DN
29	5, 16, 27	10	8	5	55	40	40	410	55.90	1 DN
30	As No. 21	8	–	10	60	40	45	325	59.20	1 DN
31	—	5	–	3	65	–	10	397	57.40	1 DN
32	After rain	1	–	3	1	–	5	390	62.25	1 HS

19 and 20, dust applied at 9- and 11-day intervals controlled the rust satisfactorily, but more evidence is needed to be sure that applications at such wide intervals will afford consistent control.

In the experiments at Crookston, 3 or more applications of Kolodust at the rate of 150 pounds per acre for each application controlled stem rust sufficiently for all practical purposes. Two applications greatly reduced the amount of rust and delayed its appearance. One dusting seemed to delay the appearance of the rust (Table 6, plots 2 and 3).

As shown in table 6 and figure 1, there was a noticeable difference in the yield of dusted and non-dusted plots. The preventable loss due to stem

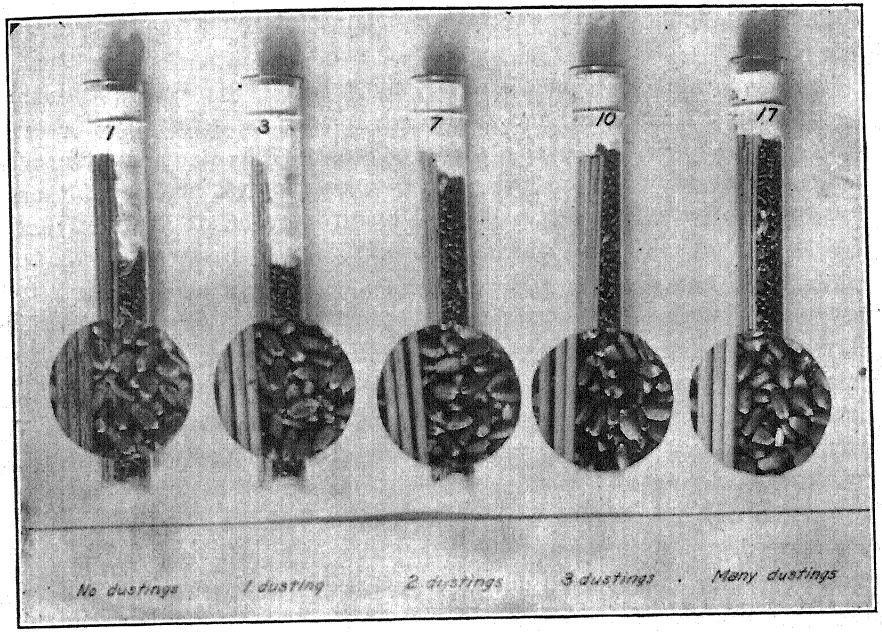


FIG. 1. The effect of sulphur dust on the percentage of stem rust and consequent yield, and quality of grain in wheat plots at Crookston, Minn., in 1926. The grain in the test tubes is the yield from 50 head samples.

Plot No.	Date dusted	Per cent of rust at harvest	Yield in bushels per acre	Grade
1	No dust applied	75	22.0	3 DN
3	July 15	70	24.5	1 DN
7	July 10, 19	40	29.5	1 HS
10	July 10, 15, 20	5	30.8	1 HS
17	July 5, 10, 15, 20, 25, 30	trace	34.5	1 DN

rust at Crookston in 1926 was approximately 25 per cent. In other words, dusting with sulphur increased the yield 30 per cent.

DISCUSSION AND CONCLUSIONS

Stem rust of wheat can be controlled by dusting the growing plants with a suitable sulphur dust. The technique should not be much more difficult than that required for dusting fruit and vegetable crops. No new principles are involved. The essentials for success are the right kind of dust and its opportune and proper application. Satisfactory dusts are available at reasonable prices; and, apparently, relatively small amounts,

properly applied, will protect wheat against destructive outbreaks of stem rust.

Two important problems, however, must be solved: Effective dusting machines must be devised, and, above all, it must be ascertained whether dusting will be profitable over a period of years. The ingenuity of manufacturers probably can be relied upon to solve the first problem, although any one familiar with wheat fields will readily appreciate the fact that it may be difficult to perfect a machine capable of dusting quickly and properly the two or three million wheat stalks in an acre of land. To dust small experimental plots thoroughly with a hand-operated duster is relatively easy; to dust larger fields thoroughly with a large machine is quite another matter. If dusting is to be a practicable method of rust control, it will, of course, depend finally on the average net profits to the farmer. In this respect the problem is no different from that involved in any other spraying and dusting problem. It would be advantageous if the onset of rust attacks and the probability of epidemics could be predicted with some degree of certainty. Whether this ever will be possible is not yet entirely clear. About all one feels justified in saying now is that even in severe epidemics stem rust can be practically and sufficiently controlled in experimental plots by dusting. Whether it can be controlled profitably by the farmers of the spring-wheat area is a question to be answered by future experiments.

SUMMARY

1. Field experiments were made at several places in the spring-wheat area in 1925 and 1926 to determine the practicability of controlling stem rust of wheat with sulphur dust. When the dust was applied properly, stem rust was effectively controlled even in the presence of severe natural epidemics.

2. Of the five different commercial brands of dust used, Kolodust was the most effective.

3. Dusting should begin when stem rust first becomes noticeable in wheat. Usually in Minnesota and North Dakota the dusting program need not be started until the wheat is in blossom.

4. The most satisfactory interval between dustings seemed to be five days. There is some evidence that rust may be controlled by dusting at ten-day intervals, but more experiments are needed to determine whether sulphur dust will adhere to wheat for this length of time in all kinds of weather.

5. Three or more dustings seem to be necessary to insure rust control sufficient for practical purposes. One or two dustings may control rust under certain conditions.

6. The most desirable rate of application has not yet been determined. Applications of 150 pounds each per acre controlled rust readily, and there is some evidence that 50 pounds or less may be sufficient.

7. In 1926 the dust treatment apparently increased the yield at Crookston, Minnesota, about 30 per cent. But even this gain would not cover the cost of three applications at the rate of 50 pounds per acre.

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DISEASES OF ECONOMIC PLANTS IN PERU[1]

E. V. Abbott

Our scientific literature contains very few reports on the diseases of economic plants in Peru. From time to time, popular articles concerning certain fungous diseases have appeared in various monthly journals published in Lima, but the writer has not been able to find any paper presenting a systematic survey of the diseases of the country. The English literature relating to the subject appears to be limited to a few papers dealing largely with the rust fungi which have been collected by botanical expeditions.

The diseases herein reported are those which have been identified by the writer since the establishment at Lima, early in 1927, of the agricultural experiment station of the Sociedad Nacional Agraria (National Agrarian Society) of Peru. While it is not expected that this is a complete list of all the diseases extant in the country, it is believed that the more common diseases of the important crops are included, since in making the survey visits have been made to nearly all of the principal agricultural sections of the country.

For the purposes of this paper, Peru may be conveniently divided into three geographical regions: the coast, the sierra, and the montaña. The coast includes the narrow, western slope of the Andes mountains, where rain never falls or is so rare as to be of no importance in agriculture. All crops, therefore, are grown under irrigation. The cultivated land of this region is limited to some twenty-five or thirty narrow but fertile valleys, each separated from the other by a stretch of barren, sandy desert. Instead of being tropical, as might be expected from its geographical position, the climate of the coastal zone is temperate, due to the cold Humboldt ocean current which flows from the South. Cotton and sugar cane are the major export crops, while rice, corn, potatoes, fruits, and vegetables are grown for domestic consumption.

The sierra comprises the plateaus and highland valleys of the Andes, the elevation of the cultivated lands varying from about 6,000 to 13,000 feet above sea level. There is a wide range of temperatures, depending upon the elevation. Although in many sections sufficient rain falls during the year for crop needs, irrigation is a common practice. Wheat, barley, corn, coca (*Erythroxylon coca*), and potatoes are the principal crops or the sierra.

The region referred to as the montaña lies east of the Andes mountains and comprises the valleys of the headwater rivers of the Amazon. The

[1] Contribution from the Experiment Station of the National Agrarian Society, Lima, Peru. Published with the approval of the Director.

elevation varies roughly from 1,500 to 6,000 feet above sea level. While climatic conditions are generally tropical, with heavy rainfall during about six months of the year, irrigation is practiced in some sections. Sugar cane, cotton, coffee, and tropical fruits constitute the crops of most importance.

The diseases included here are listed by hosts rather than according to the scientific classification of the causal organisms.

SUGAR CANE

1. *Mosaic.* The first positive identification of the mosaic disease of sugar cane in Peru was made by the writer in some plots of cane held in quarantine at Lima, in July, 1927. The following November it was found in commercial fields a short distance from Lima. A survey of the sugar haciendas of the Lima region has shown that the disease is generally distributed in this section, the infection in different fields varying from less than one per cent to 90 per cent. Counts have not shown more than 15 per cent infection with the exception of one hacienda, and fortunately the growing of cane is to be discontinued there this year. The varieties affected include Bourbon or white cane, Louisiana Purple, and some varieties of Barbados.

The mosaic was introduced into commercial cane fields in the Lima region about eight years ago and became distributed before the disease was known here. The original source of the disease is said to have been stalks of cane introduced from Argentina.

Although visits have been made to all of the principal cane-growing valleys of the coast and to three in the montaña, up to the present time neither the writer nor other representatives of the experiment station have found mosaic outside of the region of Lima.

2. *Red rot* (*Colletotrichum falcatum* F. A. Went). Red rot occurs fairly commonly in the coastal valleys, but the losses caused are seldom of great importance. Only one instance of serious loss caused by this disease has come to the writer's attention, this during the present year in an unidentified variety of cane.

3. *Eye spot* (*Helminthosporium ocellum* Faris). Although eye spot is not uncommon both on the coast and in the montaña, it is seldom of much importance. A heavy infection of Bourbon cane was observed during 1927 in the valley of Oxapampa of the montaña.

4. *Ring spot* (*Leptosphaeria sacchari* Van Breda de Haan). Ring spot is more widely distributed in Peru than the eye spot. Although the disease may be found in almost any field of cane, when heavy infections occur they are usually limited to plants in a small area. Two cases of severe leaf injury by this disease were reported during 1927, one from the coast and one from the montaña. The plants were nearly mature, however, so that only slight loss resulted.

5. *Rind rot* (*Melanconium sacchari* Mass.). This disease occurs wherever cane is grown in Peru. It is most prevalent in fields which have been badly damaged by the stalk borer.

6. *Root disease.* Cane suffering from the root-disease "complex" has been observed both on the coast and in the montaña. Bourbon cane growing in heavy soils appears to be more susceptible than varieties of Barbados. Although a study of the problem is in progress, it is impossible to state at present what factors are of most importance. In the field where root disease is present, the characteristic growth of *Marasmius* sp. is often observed, while root pitting, presumably caused by soil animals, is common. In culturing rotted cane roots in the laboratory, *Trichoderma koningi* Oud. and species of Marasmius, Rhizoctonia, and Fusarium are most frequently encountered. Pythium has not yet been isolated.

7. *Sheath disease* (*Chaetomella sacchari* Delacr.). This fungus occurs commonly on old leaf sheaths of sugar cane in Peru, but it is of no economic importance.

8. *Cold chlorosis.* During the winter months from June to December, the Bourbon cane suffers to a marked degree from the cold, particularly in the southern valleys of the coast. At this season of the year it is not unusual to see fields of Bourbon cane in which the normal green color has almost entirely given way to a sickly yellow, and on many leaves the elongate, white spots, characteristic of extreme cold chlorosis, may be found. When a field of Bourbon cane is seen growing next to one of a Barbados variety, the lesser degree to which the latter is affected by cold is very striking.

9. *Other chloroses.* A chlorotic condition of cane, apparently very similar to the Pahala blight described by Lee and McHargue,[2] has been observed several times on the coast of Peru. Plants showing this chlorosis occur in rather salty soils. There has been no opportunity to determine whether the chlorosis could be corrected by the application of manganese or iron compounds either to the soil or to the plants.

COTTON

1. *Wilt* (*Fusarium vasinfectum* Atk.). Wilt is known to occur in four valleys of the coast section and is of importance in two, the Rimac and Chancay. Tanguis, a cotton variety, originated about ten years ago by a Peruvian grower, whose name it bears, and the one most extensively grown in the country, was long believed to be resistant to the disease. If it was originally resistant, it appears to have lost this property in recent

[2] Lee, H. Atherton, and J. S. McHargue. The effect of a manganese deficiency on the sugar cane plant and its relationship to Pahala blight of sugar cane. Phytopath. 18: 775–786. 1928.

years. During 1928 the writer found fields of Tanguis with as high as 75 per cent infection by wilt. Tanguis is much more resistant, however, than Pima, Egyptian, or American Upland cotton. Infected plants of these varieties are usually killed during the first year, while the Tanguis may not show serious effects of the disease until the second season. In some instances diseased plants will continue to produce a crop for three years, although the yield is reduced.

2. *Sore shin* (*Corticium vagum* Berk. and Curt.). Sore shin or damping off of young plants occurs wherever cotton is grown in Peru, being most prevalent in heavy soils, and in cotton planted early in the spring. From five to ten replantings are necessary in some instances.

3. *Leaf spot* (*Alternaria tenuis* Nees.). This leaf disease is general on cotton in Peru. While it is usually not of major importance, a few instances have been noted where it resulted in partial defoliation of the plants.

4. *Leaf, bract,* and *boll spot* (*Helminthosporium gossypii* Tucker). This disease is widely distributed in the country, but is of minor importance.

5. *Mildew* (*Ovulariopsis gossypii* Wakef.). Mildew has been observed in the Department of Piura, extreme north coast, and in the vicinity of Lima. Heavy infections may occur in Piura on cotton growing in moist soils, causing curling of the leaves and premature falling. In 1927 the disease was rare, while in 1928 the heaviest infection in many years occurred.

6. *Boll rots.* Boll rots of cotton caused by parasitic organisms are not of importance in Peru. Where fungous infection of bolls occurs it is usually by saprophytic or weakly parasitic organisms which enter through insect punctures or other mechanical injuries. Bolls so rotted seldom exceed 10 per cent of the total crop, and the percentage is usually much less. The fungi which are found most commonly on or within rotting bolls are *Rhizopus nigricans* Ehr., *Aspergillus niger* Van Tieg., *Hormodendrum cladosporioides* (Fres.) Sacc., and *Fusarium* sp. *Diplodia gossypina* Cke. is rare.

Up to the present time anthracnose (*Glomerella gossypii* (E. A. Southworth) Edg.), or the bacterial leaf and boll disease (*Bacterium malvacearum* EFS.), has not been observed in Peru by the writer.

7. *Growth disorder.* A condition closely approximating the tomosis or leaf-cut described by Cook[3] in Haiti has been observed in the Departments of Piura and Lambayeque in the north-coast section. The trouble is characterized by the crinkled and abnormally developed leaves, the margins of which often appear to have been cut or broken very irregularly. An entire plant or only a few leaves may be affected. Young plants appear to be particularly susceptible, and, if the main growing shoot is involved, they

[3] Cook, O. F. Leaf-cut, or tomosis, a disorder of cotton seedlings. U. S. Dept. Agr., Bur. Plant Indus. Circ. 120: 29–34. 1913.

may be seriously stunted. Chlorosis of diseased leaves is not marked. Where the disease has been observed by the writer, cotton of the Upland type was affected to a much greater extent than Pima or the native Tanguis.

The observations reported here have been made while on brief trips to the sections mentioned, no opportunity having presented itself for continuous study through a growing season.

8. *Root knot* (*Heterodera radicicola* (Greef) Muell.) is known to occur in one valley of the coast.

CORN

1. *Leaf spot* (*Cercospora sorghi* Ell. and Ev.). The **Cercospora** leaf spot, widely distributed on corn in Peru, is probably the crop's most important fungous disease. It often causes a severe firing or drying of the leaves before the plants are mature.

2. *Leaf blight* (*Helminthosporium turcicum* Pass.). Although leaf blight is as of general occurrence as the **Cercospora** disease, it is of less economic importance.

Both the **Cercospora** and the **Helminthosporium** are present throughout the year on *Holcus halepensis* L., which has come to be one of the worst weeds in cultivated fields on the coast.

3. *Rust* (*Puccinia sorghi* Schw.) occurs wherever corn is grown in Peru, but it is seldom of importance. Heavy infections involving 100 per cent of the plants in a field have been observed only in the vicinity of Cuzco.

4. *Smut* (*Ustilago zeae* (Beckm.) Ung.). Smut is general on corn, but is of minor importance.

5. *Cob rot* (*Basisporium gallarum* M. Molliard). Cob rot has been observed by the writer only in the vicinity of Lima, where it may affect 10 to 20 per cent of stored corn.

6. *Seedling and root rots.* A study of these diseases is now being made. Preliminary results indicate that species of **Fusarium** are the most important causal agents. Infection of seed corn by *Fusarium moniliforme* Sheldon is common.

WHEAT

1. *Stem rust* (*Puccinia graminis* Pers.). The cultivation of wheat in Peru is at present limited entirely to the sierra, largely because of the severity with which the crop is attacked by stem rust on the coast. It is said that rust has made the cultivation of wheat on a commercial scale on the coast impossible for more than a century.

Rust also occurs in epidemic form in the sierra, but the disease develops sufficiently late in the growing season to enable the plant to reach maturity. The traveler in the sierra will often see crosses and wreaths of flowers in

the wheat fields cultivated by the Indians, placed there as a means of invoking divine protection for the crop against rust and smut.

During the past year, sixty varieties of wheat, obtained through the courtesy of the United States Department of Agriculture and of several of the state experiment stations in the United States, were tested for rust resistance at the experiment station. Only four of these, Pusa, White Federation, Kanred, and Hope produced mature grain. In the case of Pusa this was due to its earliness rather than to any ability to resist rust infection. Although the other varieties mentioned rusted heavily, a few heads were partially filled.

2. *Leaf rust* (*Puccinia triticina* Eriks.) is general on wheat in Peru and occurs in epidemic form.

3. *Covered smut* (*Tilletia laevis* Kuehn) is general in the sierra. According to information received from farmers in various parts of that region, the loss from the disease in some instances may amount to 50 per cent of the crop, but these reports have not been verified. Where the disease has been observed by the writer near Arequipa and Cuzco, the infection did not exceed 15 to 20 per cent. Seed treatment for control is seldom practiced.

4. *Loose smut* (*Ustilago tritici* (Pers.) Rostr.) is not uncommon, but is usually of minor importance.

5. *Other diseases.* The Oidium stage of *Erysiphe graminis* DC. was present throughout the season of 1928 in the fields of the experiment station. *Helminthosporium sativum* Pam., King, and Bakke has been found associated with black point of wheat grains received from the sierra. Specimens of *Fusarium culmorum* (W. Sm.) Sacc. on seedlings, and what appears to be *Rhynchosporium graminicola* Hein. on nodes of mature plants, have been received from the same section.

BARLEY

1. *Stem rust* (*Puccinia graminis* Pers.). Stem rust occurs wherever barley is grown in the country, and, while the disease usually is not so serious as on wheat, it causes a considerable annual reduction in yield. Several varieties imported from the United States during the past year suffered less from stem rust than the principal variety now grown in the country.

2. *Net blotch* (*Helminthosporium teres* Sacc.). Net blotch, general on barley in Peru, often occurs in severe form. In 1927 the barley at the experiment station farm was a failure because of this disease. During the past year field tests have been made with eighteen varieties received from experiment stations in the United States, of which the following suffered much less from the disease than did the variety at present grown here, *viz*, Oderbrucker, Heil's Hanna, Beardless 72, Horsford, Pedigree 5–1 (Wisconsin), and Daniels.

3. *Leaf scald* (*Rhynchosporium secalis* (Oud.) Davis). A leaf disease caused by a species of Rhynchosporium, probably *R. secalis* (Oud.) Davis, is general on barley and sometimes causes a severe leaf blighting.

4. *Loose smut* (*Ustilago nuda* (Jens.) Kell. and Sw.). Loose smut of barley is not uncommon, but is usually of minor importance.

5. *Powdery mildew* (*Erysiphe graminis* DC.). The conidial stage of what is believed to be *Erysiphe graminis* DC. is general on barley.

ALFALFA

1. *Leaf spot* (*Pseudopeziza medicaginis* (Lib.) Sacc.). This disease has been observed only on the coast. While it is present throughout the year, the heaviest infection begins in October and continues through November, resulting at times in almost complete defoliation of the plants. During the other months of the year it is of little importance.

2. *Leaf spot* (*Pleosphaerulina briosiana* Poll.). Although this leaf spot is general on alfalfa, it is of minor importance.

3. *Rust* (*Uromyces medicaginis* Pass.) is common, but only in rare instances does it cause damage worthy of note.

4. *Downy mildew* (*Peronospora trifoliorum* de Bary) is general on alfalfa, particularly in young stands.

POTATOES

1. *Late blight* (*Phytophthora infestans* (Mont.) de Bary) is of universal occurrence on potatoes in Peru and is the one disease of this crop for which control measures are employed. Spraying is a general practice in the coast section, while in the sierra the disease is generally attributed to the weather, and consequently no effort is made to control it. Growers in that section state that in years when cool, wet weather prevails during the growing season, from 50 to 75 per cent of the crop may be lost from "hielo," the local name for the disease, which means ice or frost. Since freezing temperatures are known to occur at times in some parts of the sierra during the growing season, the writer is unable to say how much of this loss may be due to actual freezing and how much to blight. On the coast, where the crop is sprayed, the disease is kept well in check.

2. *Powdery scab* (*Spongospora subterranea* (Wallr.) T. Johnson). Observations made by the writer and reports received from various sections, show that powdery scab is widespread in the sierra. The canker stage is of common occurrence. The disease has not been observed on the coast, its absence apparently being due to the higher temperatures prevalent in that section. In view of the fact that the coast growers import all of their seed potatoes from the sierra every year, it is only reasonable to expect

that some infected tubers would be included in the shipments and that the disease would be found on the coast if conditions favored its development.

3. *Wilt* (*Fusarium oxysporum* Schl.) is general in Peru. In the vicinity of Lima commercial fields are seldom free of the disease, and in some instances during the past season the infection was as high as 40 per cent.

4. *Virus diseases.* Mild mosaic, leaf roll, and spindle tuber. Although these diseases are common on potatoes in Peru, they are not recognized as diseases by the growers. In the coast section, where the potato crop is becoming of increasing importance, it is the common belief that the use of coast-grown tubers for seed leads to a rapid degeneration or running out of the crop, a condition attributed entirely to climatic factors. Consequently, the coast growers annually import all of their seed potatoes from the sierra.

While climatic factors may have some influence in this degeneration, the universal presence of the virus diseases leaves no doubt that they are important contributing factors. The method of choosing seed tubers at present practiced in the country offers ideal conditions for the propagation of these diseases. No plant selection is made in the field, but after the crop is harvested the tubers are separated by hand according to size and the small tubers of about one-half to one inch in diameter are set aside for seed. Although the small tubers from good hills are thus included in the seed lots, a large percentage of the product of the diseased hills is likewise set aside for propagation purposes.

In view of the fact that the important diseases of the potato on the coast are seed-borne, the question of good seed stock for this section is one worthy of careful study. Coast growers, as a rule, buy their seed potatoes in the general market of Lima without having any definite knowledge of their origin in the sierra. Studies are at present being made to determine whether tubers selected from healthy, coast-grown plants and kept free of disease, will continue to produce vigorous plants when grown for several years in the same locality.

5. Tuber rots, caused by species of Fusarium, occur commonly, especially in stored potatoes.

6. *Early blight* (*Alternaria solani* (Ell. and Mart.) Jones and Grout) is general on potatoes.

7. *Wart, Synchytrium endobioticum* (Schilb.) Perc., is known to occur in the central Andes near Jauja.

COFFEE

1. *Eye spot* (*Stilbella flavida* (Cke.) Lind.) is one of the most important fungous diseases of coffee in the Peruvian montaña. A heavy attack may result in almost complete defoliation of the plant, with the resultant loss

of the berries. The fungus also causes a rot of the berries. Fortunately such heavy infections are usually confined to a relatively few plants in scattered areas over the plantations, generally where there is excessive shade. The loss from the disease is greater in wet years.

2. *Brown spot of fruit and leaf* (*Cercospora coffeicola* B. and C.). Although widely distributed in the coffee-growing sections, this disease is of less importance than the eye spot. Its principal damage is to the fruit, infection by this fungus resulting in a partial or complete rot of the ripening berries, especially in moist and heavily shaded areas.

3. *Leaf blight* (*Corticium koleroga* (Cke.) v. Hoeh.) is general in the montaña, where it causes partial defoliation, die-back of twigs, and often death of the plant.

4. *Zonal leaf spot* (*Cephalosporium* sp.), which occurs in scattered sections of the coffee districts, is of minor importance.

5. *Wilt* (*Fusarium* sp.). A disease which exhibits all of the symptoms of the Fusarium wilts has been observed in two valleys of the montaña, but the areas involved were small. Affected plants wilt slowly, and on cutting the trunk the vascular system shows brown to black discolored areas. Two species of Fusarium have been isolated from diseased plants, but it has not yet been possible to prove their connection with the disease.

6. A leaf disease (*Colletotrichum coffeanum* Noack.) has been identified from a specimen sent from a coffee plantation on the coast.

7. *Root disease* (*Rosellinia* sp.) is of considerable importance in the Chanchamayo and Perené valleys of the interior and is known to occur in at least one other coffee district, Oxapampa. While the species of the causal fungus has not been definitely determined, the symptoms of the disease are those caused by *R. bunodes* B. and Br.

FRUITS

Although Peru affords climatic and soil conditions favorable for both tropical- and temperate-zone fruits, fruit production probably is the least developed branch of agriculture in the country. Commercial orchards of any kind are very rare, the bulk of the fruit grown for the domestic market being produced by small holders. On most of the large farms are to be found small groves with a variety of fruits which supply local needs. Of major importance among the many fruits produced in the country are avocados, citrus fruits, bananas, mangos, cherimoyas, apples, grapes, and dates.

Domestic production is insufficient to meet the demands of the local market, apples, pears, and oranges being imported in considerable quantities from the United States; bananas and pineapples from Ecuador; and apples and peaches from Chile.

AVOCADO. Of the diseases of the avocado, anthracnose (*Physalospora perseae* Dodge) is by far the most important. The distribution of the disease in the country is not definitely known, but, judging from the regularity with which it is found on fruits coming into the Lima market from various parts of the country, it appears to be widespread. In the Santa Eulalia district, one of the principal sources of Lima's supply of this fruit, anthracnose is making slow but steady headway. Scab (*Sporotrichum citri* Butl.) also is of frequent occurrence on the fruit coming into the Lima market. Blotch (*Cercospora* sp.) is common, but is of minor importance.

CITRUS FRUITS. Knot (*Sphaeropsis tumefaciens* Hedges) occurs in southern Peru on limes, but it has not been seen in other parts of the country. Dry root rot (*Fusarium* sp.) of orange has been identified from specimens received from the central coast section. The fruit rot of orange caused by *Penicillium digitatum* Sacc. may always be found in the public markets and in shipped fruit. Much of the loss caused by this fungus could be prevented by more careful handling.

BANANA. Fungous diseases of the banana are of minor importance in Peru. Anthracnose (*Gloeosporium musarum* Cke. and Mass.) is present on a large percentage of the fruit sold in Lima. Considerable rotting of market fruit is also caused by *Rhizopus nigricans* Ehr. A leaf spot (*Cercospora musarum* Ashby) is widely distributed, but is of little importance.

APPLE. Black rot (*Sphaeropsis malorum* Pk.) occurs in the vicinity of Lima, where it often causes the death of young trees. A surface bark canker (*Myxosporium corticolum* Edg.) is common, but of no economic importance. Fruit rotting caused by *Penicillium expansum* Lk. emend Thom occurs frequently in stored and shipped fruit.

MISCELLANEOUS. The following diseases and their causative organisms have been identified at different times in the field or from specimens received at the laboratory: leaf spot (*Cercospora viticola* (Ces.) Sacc.) and powdery mildew (*Uncinula necator* (Schw.) Burr.) on grape; rust (*Tranzschelia puncata* (Pers.) Arth.) and root knot (*Heterodera radicicola* (Greef) Muell.) on peach; false smut (*Graphiola phoenicis* (Moug.) Poit.) on date; rust (*Cerotelium fici* (Cast.) Arth.) on fig; rust (*Uredo cherimoliae* Lagh.) on cherimoya; and a leaf spot (*Diplodia cacaoicola* Hen.) on papaya and mango. Little information is available regarding their distribution in the country.

VEGETABLES

BEANS. The following diseases of the garden bean have been identified: anthracnose (*Colletotrichum lindemuthianum* (Sacc. and Magn.) Briosi and Cav.), from the coast; powdery mildew (*Erysiphe polygoni* DC.), from the coast; leaf spots (*Cercospora cruenta* Sacc.) from the coast, and

(*Phyllosticta phaseolina* Sacc.), general; dry root-rot (*Fusarium martii phaseoli* Burk), from the coast; stem rot (*Corticium vagum* Berk. and Curt.), from the coast; and rust (*Uromyces appendiculatus* (Pers.) Lk.), from the montaña. Of these the Corticium stem rot is of most importance. In 1927 it killed more than a hundred acres of beans near Lima.

Rust (*Uromyces fabae* (Pers.) de Bary) is common on the broadbean in the sierra, but has not been observed as yet on the coast.

TOMATO. Tomato diseases which have been identified to date, all from the coast section, are as follows: late blight (*Phytophthora infestans* (Mont., de Bary); root knot (*Heterodera radicicola* (Greef) Muell.); a fruit rot, which inoculation experiments have shown to be caused by a yeast, probably *Nematospora lycopersici* A. Schneider; and mosaic.

MISCELLANEOUS VEGETABLE CROPS. The following diseases of vegetable crops, which for the most part have been identified from the vicinity of Lima, are of frequent occurrence although usually of minor importance; leaf spot (*Alternaria brassicae* (Berk.) Sacc.), on cabbage, cauliflower, and turnip; gray mold (*Botrytis* sp.), and black stalk rot (*Macrosporium sarcinula parasiticum* Thuem.), on onion; leaf spot (*Cercospora bloxami* B. and Br.), on cabbage; leaf spot (*Cercospora beticola* Sacc.), on garden beet; leaf spot (*Septoria pastinacina* Sacc.), on parsnip; powdery mildew (*Erysiphe polygoni* DC.), on pea; and powdery mildew (*Erysiphe cichoacearum* DC.), on squash and pumpkin. The last-named disease often assumes importance.

MISCELLANEOUS DISEASES

The diseases, with their causative organisms, which have been noted on plants of relatively minor economic importance in Peru are as follows:

OATS: Loose smut, *Ustilago avenae* (Pers.) Jens., and crown rust, *Puccinia coronata* Cda.

SORGHUM: Covered kernel smut, *Sphacelotheca sorghi* (Lk.) Clint., and leaf blight, *Helminthosporium turcicum* Pass.

SUDAN GRASS: Leaf blight, *Helminthosporium turcicum*.

JOHNSON GRASS: Rust, *Puccinia purpurea* Cke., leaf blight, *Helminthosporium turcicum*, and leaf spot, *Cercospora sorghi* Ell. and Ev.

ROSE: Leaf spot, *Cercospora rosicola* Pass., rust, *Phragmidium* sp., and powdery mildew, *Sphaerotheca* sp.

WILLOW: Leaf spot, *Cercospora salicina* Ell. and Ev., and rust, *Melampsora humboldtiana* Speg.

EUCALYPTUS: Leaf spots, *Cercospora epicoccoides* Cke. and Mass.,[4] and *Macrosporium* sp.

[4] The writer is indebted to Mr. William W. Diehl, of the United States Department of Agriculture, for the identification of this species.

SUMMARY

In this paper are listed 106 diseases of economic plants in Peru, divided as follows: sugar cane 9, cotton 8, corn 6, wheat 8, barley 5, alfalfa 4, potatoes 9, coffee 7, fruits 20, vegetable crops 19, and miscellaneous plants 11. Of these diseases, 95 are caused by fungi, 5 are of virus origin and one other is believed to belong to this group, 3 are caused by nematodes, and 2 are physiological.

While it is not expected that the list includes all the diseases in Peru, it is believed that it includes those of most importance.

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THE RESPONSE OF HULLESS BARLEY TO SEED TREATMENT FOR COVERED SMUT AND STRIPE DISEASE[1]

R. H. Porter, T. F. Yu, and H. K. Chen[2]

Hulless bearded barley is a crop of considerable importance throughout the lower Yangtse valley region in China. Its adaptability to the climate and to a rotation with rice accounts for its wide usage. Crop-disease surveys in East China during the summers of 1924 and 1925 showed that hulless barley was more widely grown than the hull sorts, and that covered smut caused by *Ustilago hordei* (Pers.) K. & S. was more severe on the hulless varieties. In addition, the stripe disease caused by *Helminthosporium gramineum* (Rab.) Eriks. was generally prevalent. It was not uncommon in fields of hulless barley to find 40 per cent of the heads destroyed by covered smut and as high as 15 per cent of the plants infected with the stripe disease.

Control measures for covered smut and stripe on the hulless varieties of bearded barley have not been developed. In the United States and in Europe where extensive studies for the control of these two diseases have been carried on, the investigations have dealt only with the hull types. Using several varieties of hull barley, Tisdale, Taylor, and Griffiths (6, 7), Leukel (2), and Lambert, Rodenhiser, and Flor (1) have studied the effects of formaldehyde and numerous organic mercury compounds on covered smut. Tisdale, Taylor, and Griffiths (6) also used copper carbonate, which failed to control covered smut and apparently caused an increase in loose smut.

Preliminary trials by the senior author (4) indicated that copper carbonate might prove effective in the control of covered smut on hulless barley. Its favorable response to copper carbonate, in contrast to the response of hull barley, led to a more intensive study of the seed-treatment problem. Further, it seemed evident that covered smut was more destructive on hulless than on hull barley in East China, a condition which suggested that infection with *Ustilago hordei* might occur more readily on the former type, thereby affecting the problem of control. A closely allied problem, which has not received sufficient attention, is the effect of seed disinfectants on the yield of grain. This need of information led to an

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attempt to determine (1) the effect of seed disinfectants on covered smut and stripe of hulless barley in East China and (2) the effect of seed treatment on the yield.

The experiments to be recorded were carried on during a period of two crop years beginning in the autumn of 1925. Only hulless winter barley was used in the tests. The work was interrupted by the Nationalist Revolution in 1927, which made it necessary for the senior author to leave China. Certain phases of the project, however, are being carried on by the junior authors.

I. STUDIES WITH COVERED SMUT

Preliminary trials for the control of covered smut were begun in 1925 with seed purchased from a farmer in whose field 11 per cent of the heads were infected with covered smut. Dry fungicides used consisted of Tillantin "B," powdered copper sulphate, copper carbonate, testing 54 per cent metallic copper, and a prepared carbonate, testing 40 per cent copper. All the dusts were applied at the rate of two ounces per bushel. The liquid materials were copper sulphate (1 to 40), formaldehyde (1 to 320), Tillantin "B" (three grams per liter of water), and formaldehyde (1 to 120) at 50° C. The seed was soaked two minutes in hot formaldehyde, ten minutes in copper sulphate, one hour in Tillantin, and two hours in cold formaldehyde.

The planting plan was so arranged that every third row was a check. The rows were 16 feet long and one foot apart with 15 grams of seed per row. Seven replications of each sample were planted, making eight rows of each treatment. Before harvest, it was discovered that the soil where the plot was located was not uniform because part of it had been used as a garden. The yields, therefore, were variable, except in the case of the formaldehyde-treated seed where injury to the seed reduced the stand and the yield. The average yield of smut in the untreated rows was 6.9 per cent. With two exceptions, all of the treatments completely eliminated covered smut. Tillantin solution allowed 0.2 per cent smut, while the rows treated with commercial copper carbonate had an average of 0.5 per cent, although in the latter case all of the smutted heads, 16 in number, occurred in one row.

Tests the same season in small plots 12 x 30 feet in size were made with two dusts and one liquid treatment. The stand in all plots, except where hot formaldehyde was used, was reasonably uniform as shown by counts made in equal areas. Smut control was effective as evidenced by the following results: hot formaldehyde none, commercial copper carbonate 1.2 per cent, prepared carbonate 0.7 per cent, and the untreated 27 per cent smut. These results showed that, even with a high percentage of smut on barley, copper carbonate was able to reduce it to approximately one per cent.

Seed Treatment Trials in 1926. The studies carried on in 1926 were similar to those of 1925, except that seed was secured from three different sources and only dusts were used. In addition, nine replications were made for each treatment instead of seven. The fertility of the soil was low but more nearly uniform than in 1925. Before harvest the total number of smut-free and smutted heads per row was determined. Each row was cut and threshed separately and the weight of grain recorded in grams. The reliability of the results presented in table 1 was measured by "Students Method" in terms of odds.

The results presented in table 1 show that there is a difference in the effect of a given disinfectant on seed from different sources. For example, in one case copper sulphate reduced the smut to less than one per cent, but on two of the seed lots it allowed nearly as much as on the untreated seed. Copper carbonate and Uspulun completely eliminated smut on seed from two sources, but on seed from Kaifeng both disinfectants allowed 0.68 and 0.58 per cent, respectively. The low percentage of smut in the checks, as well as the low yields, makes it difficult to measure the relative value of the materials used, but the average effect of all four treatments on the three lots of seed is summarized below:

Uspulun gave the most effective control and the highest increase in yield on the three lots of seed, but copper carbonate was nearly as effective. Copper sulphate, in the form used in these experiments, seemed an undesirable disinfectant.

In the majority of the tests shown in table 1, the increases in yield measured in terms of odds are of some significance, but it is doubtful if these increases can be entirely attributed to smut control because the percentage of smut was always low. In some cases, however, the increase in yield was more than 25 per cent. Part of this increase may be due to the control of other barley diseases. A root rot of barley of unknown cause is common and destructive, but the number of such diseased plants was in no case excessively high, and no treatment eliminated them completely. The stripe disease was not prevalent in the seed used in these tests; therefore, the control of this disease could not have entered into the results. Inasmuch as this additional increase in yield, beyond that secured by smut control, was apparently not due entirely to the control of other diseases or to an increase in stand, it is suggested that part of it may have been due to the protection of seed in the soil from soil organisms which, on untreated seed, may invade the young seedling sufficiently to lower its vitality.

Results with Small Plots in 1926–27. Seed collected at Nanking and grown from the seed used in the fall of 1925 was used in planting the test with small plots one-half square rod in size. Each treatment was planted

TABLE 1.—*Effect of seed disinfectants on covered smut and on yield of hulless barley. Nanking, China, 1927*

Source of seed	Fungicide	Average number heads per row	Per cent smut	Yield bu. per A.		Gain bu.	Per cent increase	Odds
				Treated	Untreated			
	$CuSO_4$ Dry	242	0.87	14.7	12.7	2.0	15.8	11 to 1
	Tillantin "B"	243	0.86	13.0	13.2	-0.2	- 1.6	none
hsuchow	Copper carbonate	254	0.0	14.5	13.2	1.3	10.0	3 to 1
ıgsu Province	Uspulun	247	0.0	14.9	12.5	2.4	19.2	555 to 1
	Checks, av. of all	250	4.9		12.9			
	$CuSO_4$ Dry	328	2.16	21.0	19.0	2.1	11.0	37 to 1
	Tillantin "B"	286	1.1	20.4	17.0	3.4	20.0	216 to 1
'eng	Copper carbonate	293	0.68	19.4	17.0	2.4	14.1	30 to 1
ın Province	Uspulun	279	0.57	20.6	16.2	4.4	27.2	216 to 1
	Checks, av. of all	313	2.76		17.6			
	$CuSO_4$ Dry	251	3.3	12.6	11.7	0.9	7.7	none
	Tillantin "B"	256	0.9	12.8	10.8	2.8	26.0	416 to 1
;ing	Copper carbonate	259	0.0	13.1	10.6	2.5	23.6	100 to 1
ysu Province used had 20 pct. smut in 1926	Uspulun	254	0.0	13.5	11.9	1.5	12.6	8 to 1
	Checks, av. of all	251	5.9		11.2			

Fungicide	Av. pct. smut three seed lots	Av. increase bu. per acre	Per cent increase
Copper sulphate	2.1	1.7	11.5
Tillantin "B"	0.95	2.0	14.8
Copper carbonate	0.23	2.1	15.9
Uspulun	0.19	2.8	19.7
Checks	3.9		

in triplicate with every third plot as a check. The seed was weighed for each row, using 15 grams per row. Four dust treatments were used, namely, Uspulun, Tillantin "B," copper carbonate, and Tillantin-Trochenbeize. The results of this test are summarized in table 2.

TABLE 2.—*Effect of dust treatments on covered smut of hulless barley in plots containing one-half square rod repeated three times at Nanking, China, in 1927*

Treatment	Av. heads per plot	Per cent smut	Yield bu. per A.		Gain	
			Treated	Untreated	Bu.	Per cent
Copper carbonate	2043	0.0	13.0	11.1	1.9	17.1
Till.-Trochenbeize	2107	0.03	14.6	13.5	1.1	8.2
Uspulun	2172	0.76	16.3	16.1	0.2	1.2
Tillantin "B"	2530	1.65	20.7	18.7	2.0	10.7
Checks, av. of all	2149	5.2		14.9		

The data presented in tables 1 and 2 are comparable from the standpoint of smut control. Copper carbonate gave, on the average, the most effective control and Tilliantin the least effective, with the exception of copper sulphate which was not used in the plot test. Tillantin-Trochenbeize, used for the first time on hulless barley, gave almost as satisfactory control as copper carbonate. Tillantin "B" apparently is uncertain in its control of smut and for that reason is undesirable, even though in some cases it caused a marked increase in yield. The fertility of the soil used for this plot test was irregular as evidenced by the yields of the untreated plots. The soil was more fertile at one end than the other, which may account for the fact that dry Uspulun in the plot test gave the lowest increase in yield of any of the treatments, but in the rod-row tests it gave the highest increase where soil fertility was not so irregular.

II. STUDIES ON THE CONTROL OF BARLEY STRIPE

Experiental work for the control of barley stripe has been carried on by Leukel, Dickson, and Johnson (3) during the past five years, but, as in the case of covered smut, most of the studies have been made on hull varieties.

These authors have thoroughly reviewed the literature on barley-stripe control, so that no review is attempted in this paper. Recently, Rodenhiser (5), in summarizing his results covering a four-year period, stated that two dusts eliminated stripe entirely and four others were almost as effective. He also stated that the effect of certain dust fungicides on the yield was not yet known, but that no deleterious effects were noticed either on oats or hulless barley.

In the experiments reported in the following pages, an effort was made to make all of the tests as nearly uniform as possible. The solutions stood at room temperature at time of application. All of the wet treatments for a given plot were made on the same day and dried under the same conditions. Only one hulless variety was used. This was a winter barley of a bearded type. No solution was used more than once, and the seed was not immersed until the material was well dissolved, after which no stirring of the mixture took place. The seed was poured into the solution and after the required soak the fungicide was poured off and the grain dumped out on papers to dry. In addition, every row was harvested, weighed, and threshed, after counts for healthy and diseased plants were secured.

Experiments with Barley Stripe, 1925–26. The original seed used in all of the tests on barley-stripe control was hulless barley purchased from a farmer near the experimental fields of the University of Nanking. Observations in the field in the early summer of 1925 showed approximately 12 per cent of the plants infected with stripe. Only a trace of either covered or loose smut was present. This seed was used in all of the treatment tests in the autumn of that year. Seed from untreated rows showing 11 per cent stripe planted in a stripe-resistance-test plot was saved for seed and used in the fall of 1926 for all of the seed-treatment tests that year. The same disinfectants as well as the same planting plan used in the covered-smut experiments in 1925 were used for this test on stripe control. Unfortunately, the soil proved to be variable and, in addition, some of the rows lodged so badly that no accurate yield records could be secured. In some cases, the total number of plants could not be determined, and, for that reason, only the average number of stripe-diseased plants per row can be given. Four of the disinfectants, viz: copper-sulphate solution, dry Tillantin, and both hot and cold formaldehyde completely eliminated stripe. Of the other materials the average number of stripe plants per row was: 54 per cent copper carbonate, four; 40 per cent copper carbonate, three; Tillantin solution, three-tenths, and powdered copper sulphate, eight-tenths. The average number of stripe plants in the untreated rows was 12.

Results with Rod Rows in 1926–27. The materials used in the autumn of 1926 were both dry and liquid disinfectants. Some of them were fur-

nished by the China-Export-Import and Bank Co. Ltd. of Shanghai. All the dusts were applied in the same way and at the same rate as described in the foregoing for covered smut. Liquid treatments furnished by this company were made up at a concentration of 0.3 per cent and the seed was

TABLE 3.—*The effect of seed disinfectants on stripe and on yield of hulless barley, Nanking, China, 1927**

Treatment	Av. No. heads per row	Per cent stripe	Yield bu. per A.		Gain		Odds
			Treated	Untreated	Bu.	Pct.	
Uspulun-Dry 2 oz. per bu.	70	0.1	8.3	6.7	1.6	23.9	1999 to 1
Tillantin-Trochenbeize	70	1.7	8.6	7.2	1.4	19.4	344 to 1
Uspulun .3% Sol. 1 hr.	71	0.0	9.3	7.2	2.1	29.1	4.6 to 1
Tillantin-Nazzbeize .3% Sol. 1 hr.	70	0.7	7.9	7.3	0.6	8.2	7 to 1
Uspulun .3% Sol. 2 hrs.	70	0.0	8.6	7.4	1.2	16.2	8 to 1
Tillantin-Nazzbeize .3% Sol. 2 hrs.	69	0.3	8.5	7.0	1.5	21.4	555 to 1
Checks, av. of all	72	14.4		7.1			

* Materials used in this test were furnished by the China-Export-Import and Bank Co. Ltd. of Shanghai, China.

soaked for one and two hours respectively. Each treatment was replicated nine times and the checks occurred every third row, so that a treated row was always next to an untreated one. Each row was harvested separately and the yield determined in grams. The results of this test are given in table 3.

In table 3 it will be noted that, of the dry treatments, Uspulun almost eliminated stripe and gave a marked increase in yield, even though the highest yield in the entire plot was less than 10 bushels per acre. Tillantin-Trochenbeize was least effective in the control of stripe but materially increased the yield. Of the two solutions used, it appears that a two hours' application of Uspulun was beyond the maximum time since it did not give so high an increase in yield as the one-hour soak and was no more effective in stripe control. Tillantin-Nazzbeize, on the other hand, gave better control and a more significant increase in yield with the two-hour soak than with only one hour.

Results with Small Plots 1926–27. In addition to the rod-row tests, a series of ½ sq.-rod plots were planted October 18, 1926, in the same way as described for covered smut of barley on page 658. The soil proved highly fertile and the yield of the checks was high. The results of this test are summarized in table 4, in which it will be noted that none of the treatments eliminated stripe, but all reduced it more than 50 per cent with the exception of copper carbonate. The yield increases are quite significant, indicating that even without absolute control of stripe it is possible substantially to increase the yield on badly infected seed.

TABLE 4.—*Barley stripe seed-treatment test with plots one-half square rod in size, repeated three times, Nanking, China, 1926–27*

Treatment	Av. No. heads per plot	Per cent stripe	Yield bu. per A.		Gain in yield	
			Treated	Untreated	Bu.	Per cent
Copper carbonate	1387	5.5	54.0	44.0	10.0	22.7
Tillantin-Trochenbeize	1458	4.0	53.5	46.0	7.5	16.3
Uspulun-Dry	1401	4.0	55.6	46.0	9.6	20.9
Tillantin "B"	1387	4.0	52.3	42.3	10.0	23.6
Checks, av. of all	1382	10.8		44.6		

SUMMARY

Hulless barley, in two separate seed lots, each infested with *Ustilago hordei* and *Helminthosporium gramineum*, respectively, were treated with dry and liquid disinfectants.

Copper carbonate and dry Uspulun reduced covered smut to an average of less than one-half per cent. The copper carbonate had a different effect from that reported on hull varieties where no control was secured. No other treatments gave such consistently effective results as the two dusts mentioned above.

An average of two tests with stripe-infested seed gave 12.8 per cent stripe on untreated seed and 2.1 per cent on seed treated with dry Uspulun. In one test, copper carbonate reduced the percentage of stripe from 10.8 per cent on the checks to 5.5 per cent on the treated rows. This dust seemed less effective than liquid treatments, but it caused a high increase in yield. Soaking the seed in Uspulun solution for either one or two hours completely eliminated stripe.

The most important thing brought out in these tests is the yield response of hulless barley to some of the dust treatments. In the case of covered-smut control where yields were secured, the highest percentage of smut on untreated seed was 5.9 per cent (Table 1). In the latter case, the largest increase in yield due to treatment was 26 per cent. In another instance, shown also in table 1, where the smut infection in plots sown to untreated seed was only 2.76 per cent, the largest increase in yield was 27.2 per cent and the lowest was 11 per cent.

In table 3 the average percentage of stripe on untreated seed was 14.4 but the largest increase in yield where odds were significant was 23.9 per cent. The average increase for the three best treatments was 21.6 per cent. In table 4, the average reduction in stripe with four treatments was 6.4 per cent, while the average increase in yield was 23.4 per cent.

The increases in yield of hulless barley due to seed treatment were apparently greater than could be accounted for by the reduction of either of the two seed-borne diseases in question.

Seed treatment of hulless barley in the lower Yangtse river valley in China should become an important agricultural practice. Either dry Uspulun or copper carbonate may be used with success.

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A LEAF SPOT OF SYCAMORE CAUSED BY *STIGMINA PLATANI* (FUCKEL) SACC.[1]

C. A. APOSTOLIDES

In the fall of 1926 some diseased leaves of *Platanus racemosa* Nutt., attacked by some unknown fungus, were sent by the Rust Nursery, Pasadena, California, to the Plant Pathology laboratory of the University of California for diagnosis.

A leaf-spot disease has been known to occur in California on the leaves of *Platanus orientalis* L. (2), but to our knowledge has never been reported on the native California Sycamore, *Platanus racemosa.* The object of this investigation was the isolation and study of the causative organism of this leaf spot of *Platanus racemosa* and the determination of its relation to the fungus attacking the oriental Sycamore, *Platanus orientalis.*

In reviewing the literature of the disease of the Platanaceae the writer found that a leaf spot was reported by von Thümen (2) in 1871 and Saccardo (3), (4) in 1878 and 1880.

The symptoms of the disease under investigation are as follows: Scattered spots 1 to 3 mm. in diameter are found on the lower surface of the leaves. (Fig. 1, B.) When severely infected, the entire lower surface is black (Fig. 1, A), and the upper side shows extensive brown areas. When the leaves are not so heavily infected, a discoloration ranging from light to dark brown appears on both sides.

The disease is characterized also by a loosening of the epidermis from the affected areas. Directly beneath such areas are found the superficial bodies of the pathogene (Fig. 2).

The surface of diseased leaves was disinfected by placing them in a 1 to 1,000 solution of $HgCl_2$ for one minute. They were then removed, passed through several changes of sterile distilled water, and allowed to remain in each for five minutes. After surface disinfection, the diseased tissues were placed in a test-tube containing 2 cc. of sterile distilled water and crushed into small bits with a sterile metal rod. Thus a suspension of spores was made containing only one to two spores per loopful. Transfers were made from the last dilution to thin, clear agar plates and examined under the microscope.

[1] Submitted as partial fulfilment of the requirements for the degree of Master of Science, University of California.

The writer wishes to acknowledge his indebtedness to Professor W. T. Horne, under whose direction the work was conducted, for valuable suggestions and criticisms, and to Dr. T. E. Raulins for suggestions, criticisms, and encouragement.



FIG. 1. *A* shows the underside of a lightly infected leaf of *Platanus racemosa*. *B* shows the underside of a severely infected leaf of *Platanus racemosa*.



FIG. 2. Photomicrograph of a cross-section of a leaf showing distinctly an emergence of conidiophores and their conidia. (The leaves were imbedded in paraffin and the sections stained with Flemming's triple stain.)

After the fungus was obtained in pure culture, it was noted that on artificial media the organism grew very slowly and rarely produced spores. The following solid media were employed: Cornmeal agar, nutrient Czapek agar, plain agar (made by adding 20 grams of agar to 1,000 cc. of water), prune, potato, *Platanus racemosa*—leaf-decoction agar, potato plugs, carrots, rhubarb, and stems of white clover and California Sycamore, *Platanus racemosa,* bran corn, and steamed rice in 200 cc. Erlenmeyer flasks. These media were made according to Duggar (1, pp. 9–40). All carbohydrate media were sterilized for 30 minutes in autoclave at 7 to 10 pounds pressure. The fungus was grown in each medium at six different times. Detailed observations were recorded daily. Of all the media tried, Czapek's synthetic agar gave the best results. On this medium the fungus grew well and produced few spores. Czapek's agar was prepared as follows: Magnesium sulphate ($MgSO_4$) 0.5 gm., potassium chloride (KCl) 0.5 gm., monobasic potassium sulphate (KH_2PO_4) 1.0 gm., sodium nitrate ($NaNO_3$) 2.0 gms., iron sulphate ($FeSO_4$) 0.01 gm., sucrose 30.00 gms. in 1,000 cc. distilled water.

Diseased leaves of *Platanus racemosa* from Pasadena were imbedded in paraffin, sectioned, and stained with Flemming's triple stain. The slides disclosed an abundant growth of intracellular mycelium in the discolored portions of the leaves and tufted conidiophores (Fig. 2), with conspicuous

one- to three-septate, ovoid to elongate, olive green, terminal conidia, 15.0 μ long × 8.9 μ wide.

In order to determine the pathogenicity of the organism the following experiments were carried out.

Surface Inoculations: Spores and mycelium were applied with a brush to the lower surface of the leaves. To prevent drying, the twig bearing the inoculated leaves was covered with a wax-paper bag containing some moist cotton.

Puncture Inoculations: The second experiment was similar to the first, except for the injury caused by pricking the leaves with a scalpel. After inoculation, the leaves were covered with mason jars containing moist cotton and wrapped with dark paper. Checks, similarly prepared, accompanied the inoculation experiments, but with the fungus omitted. Infection resulted from both of these methods, as shown in table 1.

TABLE 1.—*Infection results obtained from inoculations of leaves of Platanus racemosa with Stigmina platani*

Date of inoculation	Trees inoculated	Method of inoculation	Date of first symptoms	Inoculated			Control	
				Number of leaves inoculated	Number of leaves infected	Per cent infection	Number of control leaves	Number of leaves infected
March 17, 1927	Tree No. 1	puncture	April 21, 1927	15	6	40.0	3	None
April 27, 1927	Tree No. 1	surface	June 18, 1927	19	9	47.3	3	do
June 2, 1927	Tree No. 1	surface	June 23, 1927	10	9	90.0	3	do
March 17, 1927	Tree No. 2	puncture	April 21, 1927	18	4	22.2	3	do
April 27, 1927	Tree No. 2	surface	June 18, 1927	15	3	20.0	3	do
June 2, 1927	Tree No. 2	surface	June 23, 1927	10	10	100.0	3	do
Total				87	41	47.1	18	None

The symptoms obtained from artificial inoculations were identical with those found under field conditions. The highest percentage of infection was obtained in June. This may have been due to the exceptionally warm

weather of that month. The inoculations made on March 17, 1927, resulted in distinct dark spots within five weeks, while inoculations made on April 27 and June 2, 1927, gave similar spots in three weeks. The inoculation experiments conducted in the greenhouse and in the open gave similar results, and the organism was identified as *Stigmina platani* (Fckl.) Sacc. (*Stigmella platani* (Fckl.). The fungus was twice reisolated from artificially infected leaves.

Influence of light on growth and sporulation of the fungus: In order to determine whether light influenced the growth of the fungus, cultures were kept a month in test-tubes of different media in the dark at room temperature. At the end of this time, they were examined and compared with cultures kept at room temperature but in diffuse light. No appreciable difference was noted in the growth of the organism on any of the media used. Thus it appears that diffuse light has little if any influence on the growth of the fungus.

SUMMARY

Studies were made of a leaf spot of the native California Sycamore, *Platanus racemosa.*

A fungus was isolated, the disease was produced by inoculating leaves with the isolated organism, and the fungus was reisolated from the artificially infected leaves.

Czapek's agar was found to be the most favorable medium for the growth and sporulation of the fungus.

Studies of the morphology of the organism show it to be *Stigmina platani* (Fckl.) Sacc. (*Stigmella platani* Fckl.), an organism which causes a leaf-spot disease of *Platanus orientalis.*

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THE LIFE HISTORY OF SPHACELOMA AMPELINUM DE BARY[1]

C. L. SHEAR

The fungus, commonly known as *Sphaceloma ampelinum,* is the cause of a serious grape disease in the eastern United States and also in Europe. The disease, usually called anthracnose, sometimes causes heavy losses to both vinifera and American grape varieties. It attacks foliage, fruit, and canes. The disease appears to be of European origin and has been known for many years. According to Viala, specimens collected in 1839 are preserved at Montpellier, and it is believed that a disease of the vine mentioned by Theophrastus and Pliny is anthracnose. De Bary (3), in 1873, investigated the disease and gave an accurate description of the conidial form of the fungus, naming it *Sphaceloma ampelinum.* The earliest report of its appearance in this country, so far as we know, is that by the late Professor Burrill (7), of Illinois, who says he found it in that state in 1881. Since that time the disease has been the subject of numerous studies by different investigators in this country and Europe. As a result, the fungus has received several different names. Comes (8, p. 425) gives as synonyms *Torula meyeni* Ber. & Trev. and *Ramularia meyeni* Gar. & Catt. According to Saccardo, Passerini named it *Ramularia ampelophaga.* A little later Saccardo transferred it to the genus Gloeosporium. In 1904 and 1905, Viala and Pacottet (14, 15), in an elaborate series of papers on diseases of the vine gave an account of their cultural studies of the organism. According to their investigations the organism is extremely pleomorphic as well as polymorphic, producing two or three conidial stages; a yeast form with an ascospore stage, giant cells, resting spores, and mono- and polysporous cysts, as well as pycnidia and spermagonia. As a result of their studies they concluded that the organism should have a new generic name and they rechristened it *Manginia ampelina,* V. & P., referring it provisionally to the Sphaeropsideae and stating that when the perithecial form was found it could be put in its proper place among the Pyrenomycetes.

The writer has studied this organism in the field and in culture for many years, but has never been able to obtain in pure cultures all the forms described by Viala and Pacottet. The conidia bud readily in culture, producing forms resembling those which they describe and figure as yeast. We also find in the mycelium bodies corresponding to their cysts (Fig. 1). These are very variable in form, size, color, and septation and sometimes have the appearance of containing spores. We have never found the other

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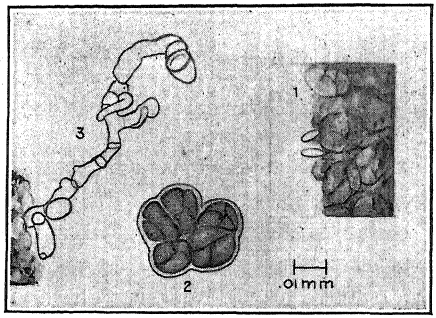


FIG. 1. *Elsinoe ampelina.* 1. Mass of cyst-like bodies bearing conidia. 2. Mature cysts with contents divided. 3. Cyst-like bodies in early stage of formation. All from pulp of an old grape destroyed by anthracnose.

forms of conidia which they figure nor the pycnidia and spermagonia which they describe and illustrate. We have, however, obtained in cultures made by transplanting diseased tissue of anthracnose spots on grapes pycnidia of a Phoma corresponding to the pycnidia which they describe. All such cultures, however, were shown to be impure when single conidia of the anthracnose fungus were isolated and grown separately. As a result of a study of closely related organisms such as *Gloeosporium venetum* Sacc. and the citrus scab organism we are led to suspect that their cultures were impure.

Early in our work on small fruit diseases we noted the striking similarity between cultures of *Gloeosporium venetum* Sacc., the fungus causing anthracnose of raspberries and blackberries, and those of the grape anthracnose fungus. In fact, the similarity is so close as to suggest the specific identity of the organisms.

In 1914 Burkholder (4) published an abstract and in 1917 (5, 6) the complete results of his studies of *Gloeosporium venetum* in which he showed that it is the conidial form of an ascomycete which he referred to the genus Plectodiscella of Woronichin (16). It at once occurred to us that the grape anthracnose fungus probably had a similar perfect stage, and this probability was suggested in a paper on grape anthracnose presented by the writer (13) at the International Congress of Viticulture in San Francisco in 1915. A few years later a serious outbreak of anthracnose in a vineyard near Norfolk, Va., came to our attention and provided an opportunity to secure an abundance of fresh material. Failing in all of our efforts to obtain the perfect stage of the fungus in culture, a quantity of severely cankered canes and shoots was obtained and kept out of doors over winter at Washington. In the spring a careful examination of these old cankers showed ascocarps very closely resembling those described and

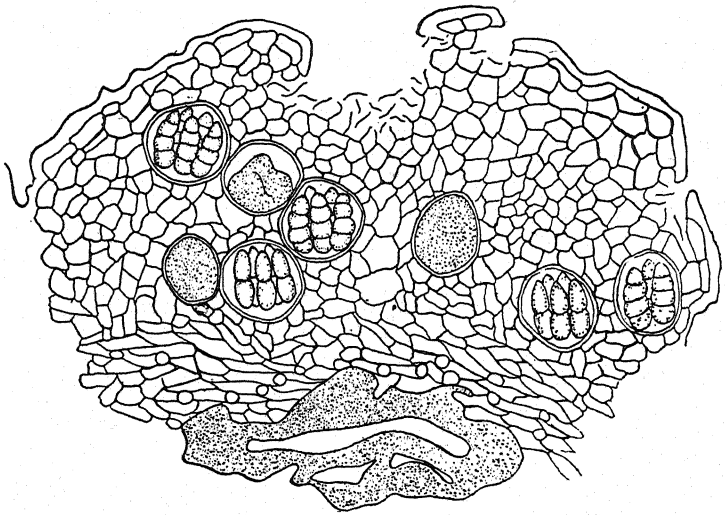


FIG. 2. *Plectodiscella veneta.* Section through an ascocarp, showing asci and ascospores × 850 (after Burkholder).

illustrated by Burkholder for *Plectodiscella veneta.* (Fig. 2.) Later some of these ascocarps showed nearly mature asci and ascospores. The ascospores are hyaline, 3-septate, and 15–16 x 4–4.5 μ; perhaps somewhat larger when fully mature. (Fig. 3.) Unfortunately, we were unable to get single ascospore cultures from this material, either because the spores were immature or the cultural conditions unfavorable. The evidence therefore of the genetic relation between *Sphaceloma ampelinum* and the ascocarps found in the cankers is at present circumstantial, being based upon the close association of the two forms in the cankers and the sequence of development and also the fact of the very close similarity between the Rubus anthracnose organism in both its stages and the grape anthracnose fungus. In fact, it is difficult to separate the two organisms on very satisfactory cultural or morphological characters in either the conidial or ascogenous stage. They are, however, at least physiologically distinct, as Dr. Anna E. Jenkins, of the Office of Mycology and Disease Survey, has shown by careful inoculation experiments (unpublished) that the raspberry anthracnose fungus will not infect the grape.

This fungus belongs to a very interesting group of ascomycetes apparently to be included in the order Myriangiales. It is characterized by ascocarps consisting of mostly pseudoparenchymous tissue in which the asci are irregularly distributed and imbedded (Fig. 3).

The genus Plectodiscella described by Woronichin (16) in 1914 and based upon *P. pyri* Wor., which causes a leaf spot of apple and pear in

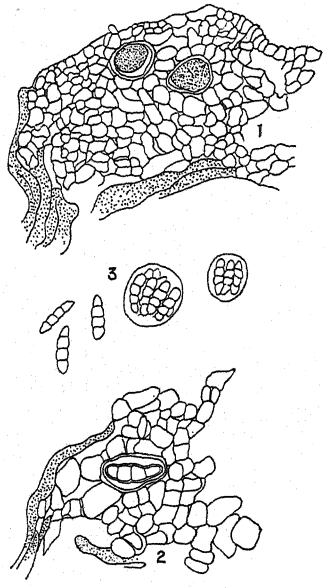


FIG. 3. *Elsinoe ampelina*. 1. Vertical section of an ascocarp with young asci. 2. Portion of an ascocarp with one ascus and nearly mature spore. 3. Asci and ascospores. From old anthracnose canker on grape shoot.

the Caucasus, has a very similar ascocarp, but more regular in outline and with a rather definite dark colored outer covering (Fig. 4), whereas the anthracnose fungus apparently has no distinct superficial layer. Arnaud (1), 1925, in discussing this and closely related genera, adopts the generic name Uleomyces of Hennings and gives as synonyms thirteen generic names, including Plectodiscella. The type of Uleomyces, *U. parasiticus* P. Henn., would hardly be regarded by most mycologists as congeneric with the type of Plectodiscella. *Uleomyces parasiticus* P. Henn. (*U. sanguineus* (Speg.) v.H.) as shown by Arnaud's (1) description and figures has a superficial stroma and many septate, muriform, brown ascospores, instead of hyaline 3-septate spores, as in the type of Plectodiscella. One of the genera which Arnaud regards as a synonym of Uleomyces is Elsinoe of Raciborski (11). The type of this genus is *E. canavalliae* Rac. 1900 (Fig. 5). From Arnaud's detailed description and illustrations of the type collection of this species, it is very clear that Elsinoe is a synonym of Plectodiscella, unless the dark colored superficial cover in the latter be considered a distinctive character, and that the type species of Elsinoe is very similar in all of its characters to *P. veneta* on Rubus and to the species on grape. On the basis of priority therefore the generic name of the apple, Rubus, and grape fungus should be Elsinoe, Raciborski, 1900, instead of Plectodiscella, Woronichin, 1914. We would therefore refer the ascogenous

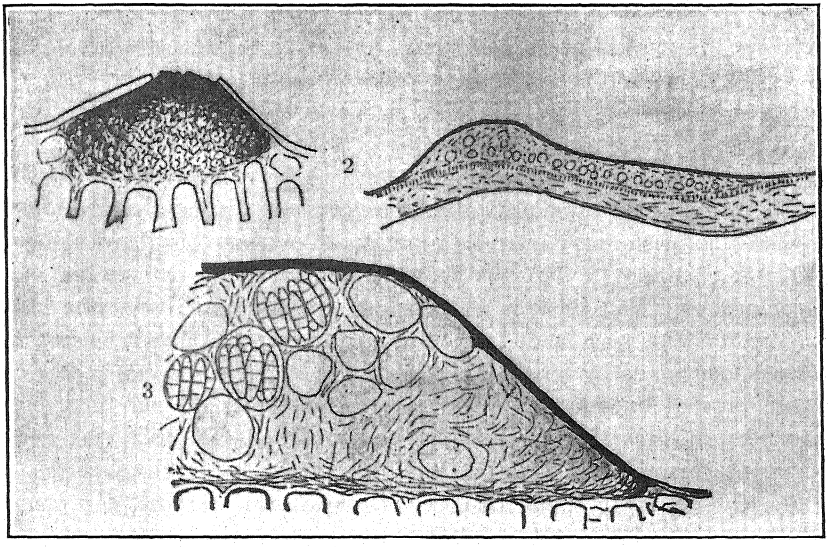


FIG. 4. *Plectodiscella pyri.* 1. Section through fundament of a stroma. 2. Young stroma. 3. Section through a mature ascocarp. (1, 3 × 500; 2 × 82; after Woronichin.)

form of the grape anthracnose organism to the genus Elsinoe as *E. ampelina* n. sp. In this connection it should be mentioned that Raciborski (12) described a species of Elsinoe which he collected in Java as *E. viticola.* It was found on leaves of a species of Vitis. His description of the asci and spores agrees very closely with our grape fungus, and it is possible as Jenkins and Horsfall (10) suggest that *E. viticola* Rac. is a synonym of *E. ampelina* (de By.) Shear, **n. comb.** Arnaud, however, states that he was unable to find any good ascocarps of *E. viticola* on the specimens from Raciborski preserved in the Paris Museum.

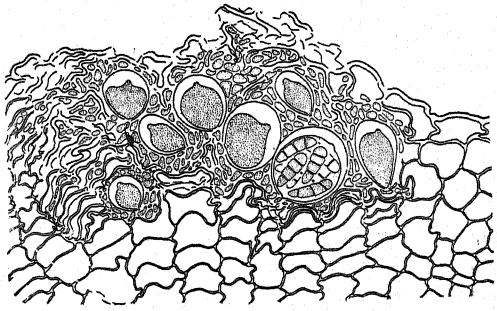


FIG. 5. *Elsinoe canavallae* Rac. Section of an ascocarp showing asci and ascospores (after Arnaud).

It is interesting to note in connection with the life history of these organisms the remark made by Arnaud (1) in his reference to *Plectodiscella veneta* Burk., p. 688. In speaking of the genetic relation between *Gloeosporium venetum* and Plectodiscella, as shown by Burkholder, he says, it is more probable that the Plectodiscella is a parasite of the Gloeosporium. This remark was apparently suggested by the fact that most of the species of this group appear to be parasites on other fungi, rather than by a careful consideration of the very convincing evidence produced by Burkholder.

Doctor Jenkins (9, 10) has shown that the citrus scab organism is a Sphaceloma and that there is also one on Avocado and rose. She has also found on the type specimens from Woronichin conidia of Sphaceloma which were not mentioned by him in his description of *Plectodiscella pyri*.

Our present knowledge of the ascogenous stages of conidial forms of Gloeosporium furnishes an excellent example of the great diversity of ascogenous forms having similar conidial stages, *e.g.*, *Pyrenopeziza ribis*, conidia, *Gloeosporium ribis; Glomerella cingulatum*, conidia, *Gloeosporium cingulatum; Plectodiscella venetum*, conidia, *Gloeosporium venetum*. On the other hand we have such cases as Physalospora and Botryosphaeria with very similar ascocarps but with such different pycnidial forms as Sphaeropsis and Dothiorella as their respective imperfect stages.

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THE EFFECT OF *TILLETIA CARIES* (DC.) TUL. (*T. TRITICI* (BJERK.) WINT.) ON THE DEVELOPMENT OF THE WHEAT EAR

W. A. R. Dillon Weston, M.A.

In the course of investigations carried out at Cambridge during the past six years on the relative resistance and susceptibility of wheat varieties to bunt, the writer has made observations on the morphological changes which take place in infected ears of a large number of wheat varieties.

Figure 1 is the variety American Club (*T. compactum* Host.). The ear on the left is bunted, and the one on the right is clean. It will be seen that

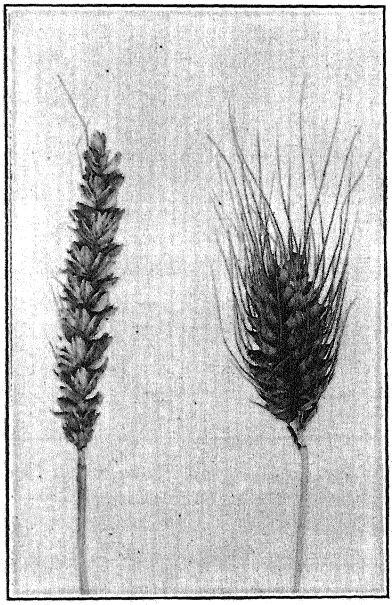


Fig. 1 is the variety American Club (*T. compactum*). The ear on the left is bunted, the ear on the right is clean.

some of the spikelets on the bunted ear are awnless. En passant it is interesting to note the elongation of the ear in this case. This, although not invariably occurring in *vulgare* types, is very general; in *compactum* types, however, in our own experiments with winter-sown American Club it has invariably occurred, and the bunted *compactum* head is then very similar to a clean *vulgare* variety. Appel (1, 2) and Edler (3) have

previously discussed this extreme difference and it appears that exceptions occur more frequently in spring-sown club. Such marked elongation has not been noted in other species which have been under observation:—viz, *T. sphaerococcum* Perc., *T. Spelta* L., *T. polonicum* L., *T. durum* Desf., *T. dicoccum* Schr., *T. persicum* Vav. and *T. turgidum* L.

Figure 2 shows the variety Velvet Don (*T. durum*). The bunted ear is on the left and the clean is on the right. There is again a total suppression of awns.

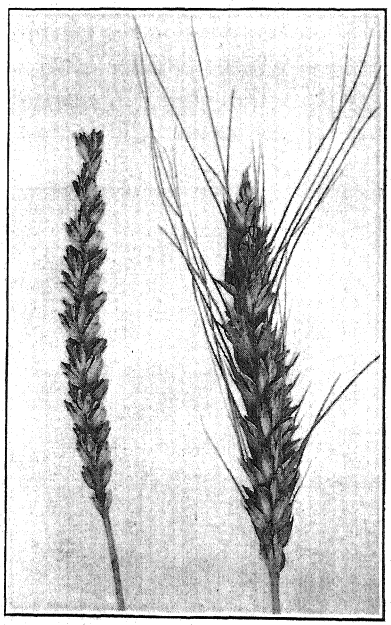


FIG. 2 is the variety Velvet Don (*T. durum*). The ear on the left is bunted, the one on the right is clean.

Figure 3 shows the variety Turkey (*T. vulgare*). The bunted ear is on the left and the clean, on the right. In this case also there is a total suppression of awns.

Figure 4 is the same variety. Three ears on the left have awns and three ears on the right are awnless, but the specimens on both the right and left are bunted.

The following explanation of this phenomenon is offered. The ears on the left were harvested two or three weeks before those on the right. In the first case they were cut when immature and still green, but in the second case they were cut when ripe. This "suppression" of awns is therefore clear. It has no genetical significance, and is due to the awns break-

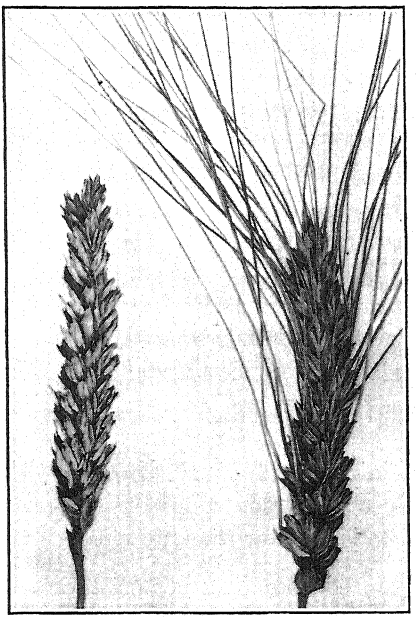


FIG. 3 is the variety Turkey (*T. vulgare*). The ear on the left is bunted, the one on the right is clean.

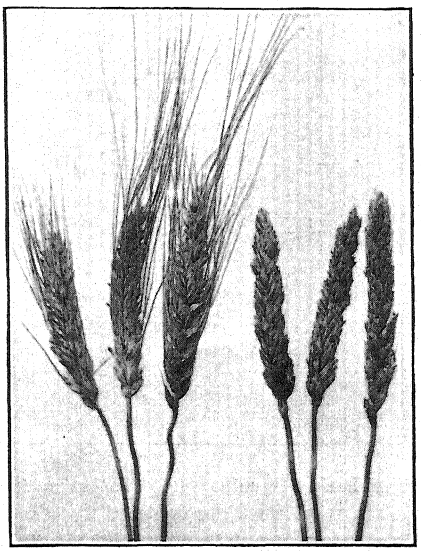


FIG. 4 is the variety Velvet Don, awned and awnless. Each specimen is bunted, but the awned specimens were harvested before they were ripe.

ing off when the culms swayed from side to side. It may be argued that in these cases why did the awns of healthy ears not break off? It is suggested that parasitism of this nature will inevitably result in a weakness in the host; for example, infection with bunt in the majority of cases increases the susceptibility of a variety to yellow rust (4) (6). Moreover, the life history of the host will be prematurely terminated since, in consequence of the complete parasitism, the last remaining sources of food will be required to nourish the parasite in the production of its countless progeny.

Figure 5 is the variety Marshal Foch (*T. vulgare* Vill.). The plate shows two ears, the one on the left is bunted, the one on the right is clean. In the case of this variety where the normal awns exhibit a small development only it will be noted that the awns in the bunted ear are not suppressed.

Murashkinski (5) in a recent paper discusses these modifications and draws attention to the suppression of awns in normally awned varieties. The purpose of this note is to show the true nature of this suppression.

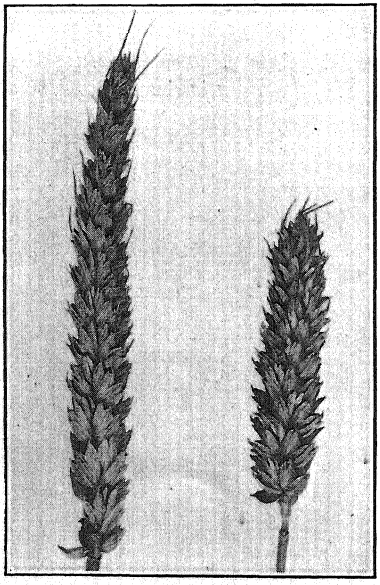


FIG. 5 is the variety Marshal Foch (*T. vulgare*). The ear on the left is bunted, the one on the right is clean.

The writer is indebted to Mr. C. W. Williamson for the photographs of these wheats.

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PHYTOPATHOLOGICAL NOTES

Report of the Cotton Root-Rot Conference at College Station, Texas.—The root-rot disease, caused by *Phymatotrichum omnivorum* (Shear) Duggar (*Ozonium omnivorum* Shear), is one of the most destructive plant diseases known and is of tremendous importance in the agriculture of the Southwest. In 1927, a much expanded program of investigation at the Texas Agricultural Experiment Station was made possible through special appropriation of $100,000 by the legislature for basic research on root-rot during the following biennium. This work has been carried on at College Station and at the Temple, Iowa Park, and Weslaco substations. More recently, Congress has also made special appropriations for work on root-rot, to be carried on by the Bureau of Chemistry and Soils with headquarters at Austin, and the Bureau of Plant Industry with headquarters at Greenville. These various research agencies have recently entered into a cooperative agreement for the purpose of obtaining maximum results in the study of the cotton root-rot problem with the facilities available.

The purpose of the conference, called by Director A. B. Conner of the Texas Agricultural Experiment Station and held at College Station on January 14 and 15, 1929, was to bring together the workers with root-rot from these various organizations, determine the present status of knowledge, and plan for future investigation. Dr. J. J. Taubenhaus, Chief of the Division of Plant Pathology and Physiology, and in charge of the root-rot research of the Texas Experiment Station, presided. The scope of the discussions of results obtained in the various studies of root-rot may be indicated from the headings of the program: Description, distribution, and importance; the fungus; infection and wintering-over problems; environmental relations; control; discussion of future work.

Some significant results presented at the meeting by the Texas Experiment Station workers were as follows: *Phymatotrichum omnivorum* is greatly inhibited in growth at pH 6.0 whether this acidity is produced by any of three different acids.[1] In an extensive survey, root-rot was found in only 34 per cent of fields with even slightly acid soils and in 71 per cent of fields with alkaline soils and was not destructive even when present in acid soil. Seven different soils were used in an experiment in which each soil was placed in a long box, a row of cotton planted along the middle, and one end of this row inoculated. Spread of root-rot correlated directly with the pH of the soil, and infection did not occur in the two more acid soils.

[1] A portion of these results is given in: Taubenhaus, J. J., W. N. Ezekiel, and D. T. Killough. Relation of cotton root rot and Fusarium wilt to the acidity and alkalinity

In three additional series of experiments at College Station during 1928, the incidence of root-rot, its spread, and the number of plants killed, were again found to be influenced by the acidity of the soil. In artificial inoculations in a long field plot, nearly perfect results were secured along the greater length of the rows, while at one end most of the plants remained normal. Again, the soil was found to be more acid where the plants remained uninfected. From these different lines of work, it was concluded that the reaction of the soil has a marked influence on root-rot and that this disease may perhaps be controlled by changing the soils to the proper acidity.

Preliminary experiments with soil acidity as a control measure were reported. Eight commercial sulphur materials were tested in containers at College Station. Plants killed by root-rot were reduced from 40 per cent in the checks to 3 per cent, 1 per cent, and 0, with the addition of large amounts of sulphur. In preliminary field tests during the season of 1928, the addition of sulphur was found of little value in soils that contained large amounts of lime; while with soils that were only slightly alkaline or neutral there was marked reduction of loss. Cotton is very susceptible to sulphur injury which occurred both in field and container experiments when heavy applications were made. It was emphasized in discussion that soil acidity as a control measure is still in the experimental stage, would have to be controlled very carefully to prevent injury to cotton, and would be applicable only to the portions of the Southwest where the soils are not so strongly alkaline as to make acidification prohibitive in cost. However, millions of acres of land in Texas were shown to be of types probably suited to this treatment.

Another line of approach was with regard to the factors underlying the rotation and clean-culture method of control, which remains the method to be recommended in the alkaline "black lands" where cotton root-rot is very destructive. Experiments were reported in which soil from the advancing edges of root-rot spots was sifted and placed in large containers. Root-rot did not attack cotton planted in this sifted soil, while in check containers of unsifted soil (which included the roots of plants) there was always root-rot. In fields, it was found that the diseased roots of the first plants, naturally infected in spring, could be traced back to roots in the ground that had over-wintered and were carrying root-rot. Numerous perennial weeds were found carrying root-rot over winter on their roots. In inoculation experiments with infected cotton roots, root-rot could not be transmitted by roots of plants that were dead; while high percentages of infection were obtained when living roots infected with root-rot were used as inoculum. These results support the tentative conclusion that root-rot lives over winter

chiefly on the living roots of susceptible plants and not in the soil itself; and suggest that, if perennial weed carriers are kept down by proper cultural methods, control may be secured with rotations that allow the roots of susceptible crop plants to die off before the next susceptible crop is planted. Studies on cotton roots have shown that many remain alive in the soil over winter and gradually die off during the following summer and fall. Similar studies are in progress with the roots of susceptible weeds.

Tests for varietal and individual resistance in cotton have been carried on at the Temple substation. During the past season, 5200 selections were included in the artificial inoculations and little resistance was found. Dr. R. B. Streets, however, stated that he had selected Pima and Acala strains which appeared to be resistant.

Dr. D. C. Neal, of the U. S. Department of Agriculture, reported that he had been able to confirm observations previously made by Messrs. C. J. King and H. F. Loomis, also of the U. S. Department of Agriculture, and had obtained true sclerotia of *Phymatotrichum omnivorum* in artificial culture, in addition to the pseudosclerotia previously known. Dr. Taubenhaus described the production of a spore mat (the Phymatotrichum stage) in a box containing plants artificially inoculated with root-rot.

Results of the work of the Bureau of Chemistry and Soils during the past season were presented by Dr. Oswald Schreiner and Dr. Paul R. Dawson. Preliminary fertilizer experiments yielded inconclusive results. Root-rot was found to occur only in the less acid part of a field in which it was distributed unevenly.—W. N. EZEKIEL, College Station, Texas.

Leptosphaeria foot-rot of wheat in Alberta.—In addition to *Helminthosporium sativum* P. K. and B., *Ophiobolus graminis* Sacc. and *Fusarium* spp., all of which cause foot-rots of wheat in Alberta, another organism has been isolated from wheat plants affected with foot-rot. It was first obtained from wheat stubble collected at the University of Alberta in October, 1927. In has since been obtained from various parts of the Province. Perithecia of this fungus have been found in abundance on old wheat stubble. In one case numerous perithecia were found on wheat stubble of the first crop after breaking and a similar fungus was collected on wild grasses surrounding the field.

The fungus proved to be a species of Leptosphaeria, the ascospores of which closely resemble those of *Leptosphaeria herpotrichoides* de Not., one of the most important cereal foot-rotting fungi of continental Europe. The ascospores measure 25–40 μ (33 ± 2) x 4–6 μ and are 6 to 8-septate. In the specimens so far examined, however, the ostioles are less raised and the perithecia lack the hairiness described for *L. herpotrichoides*. In the latter

respect our fungus more nearly resembles *L. culmifraga* Fries (Ces. and de Not.), which, according to Saccardo,[1] scarcely differs from *L. herpotrichoides* except in its less villose perithecia. According to Mangin[2] these characters of the perithecia vary widely in different collections of *L. herpotrichoides*. Their development is probably greatly influenced by environmental factors. Another characteristic in which our fungus corresponds to *L. herpotrichoides* is in the production of "ampoules perforatrices" or appressoria described by Mangin,[2] Foëx,[3] and others as typical of this species. These were produced abundantly on Marquis wheat artificially inoculated with a monosporous culture.

The fungus did not cause seedling blight like *Helminthosporium sativum* and *Fusarium graminearum* Schwabe, but attacked the plants later, in this respect resembling *Ophiobolus graminis*. Definite foot-rot symptoms were produced in laboratory and greenhouse tests. The optimum temperature for vegetative growth apparently lies between 20 and 25° C., thus corresponding more closely with *Ophiobolus graminis* than with the other above-mentioned foot-rotting fungi. Specimens submitted to the Imperial Bureau of Mycology were kindly compared with herbarium material by E. W. Mason, to whom we are indebted for an excellent report.—A. W. HENRY and W. R. FOSTER, University of Alberta, Edmonton, Canada.

A fruit spot of tomato caused by Aplanobacter michiganense.—A very characteristic fruit spot of tomato, found in Georgia in 1928 and 1929 and in Mississippi in 1929, in fields where bacterial canker was present, has been definitely proved to be another phase of this disease caused by *Aplanobacter michiganese* E. F. Sm.

The spots are unlike those produced by *Bacterium vesicatorium* Doidge. They are round, never dendritic; are white, at first; later, have a lenticular rift or round, light brown, roughened center surrounded by a white halo; are never water-soaked; and may fuse to form large, roughened patches. In late stages they are dark brown but, even then, often retain the white halo.

Aplanobacter michiganense has been isolated repeatedly from natural infections, used to produce fruit spots like those from which they were isolated and reisolated from these spots. Also typical systemic infection has been produced with fruit-spot isolations and fruit-spot with isolations from typical systemic lesions.—MARY K. BRYAN, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

[1] Saccardo, P. A. Sylloge Fungorum. Vol. 2. Patavii. 1883.

[2] Mangin, L. Sur le piétin ou maladie du pied du blé. Bul. Soc. Mycol. France 15: 210–239. 1899.

[3] Foëx, E. Observations sur le piétin du blé. Ann. Serv. Épiphyties 6: 200–213. 1919.

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ETIOLOGY OF THE PINK-ROOT DISEASE OF ONIONS

H. N. HANSEN

INTRODUCTION

The name "pink root" appeared in the literature for the first time in 1917 (7), when Taubenhaus published a preliminary report followed by partial descriptions of the disease in 1918 and 1919 (8, 9) and a full account of extensive experiments on etiology and control in 1921 (10). He considered the causal agent to be a species of Fusarium and named it *F. malli* Taub. Sideris (5, 6) concluded that the disease was caused by several species of Fusarium and not by *F. malli* alone.

ECONOMIC IMPORTANCE

Definite data on economic losses from the pink-root disease are not available but it is a common observation in California that where onions are grown in the same field for two or three seasons the crop frequently is so diminished that no attempt is made to harvest it. Such conditions are commonly observed in the principal onion-growing sections of the State, in the vicinity of Stockton.

SYMPTOMS

Macroscopic Symptoms. The affected roots are characteristically pink, the shade varying with the severity and age of infection. Thus, shortly after infection has taken place, the color of the roots is a rhodonite pink (3) which, as the disease progresses, deepens through the various shades of pink to spinel red and rarely to Tyrian purple. In early stages the pink color alone is the criterion of infection but, as the disease progresses, the roots lose their turgidity and assume a semi-transparent, water-soaked appearance. At this stage, the epidermal layer and the vascular elements may be easily separated as all the intervening tissues have been disintegrated. When roots are in this condition they are easily invaded by other members of the soil flora but especially by bacteria and many species of Fusarium, as shown by the writer's[1] early isolations and by those of Sideris (6).

[1] Since the above article was accepted for publication Sideris' thesis (6) has appeared in this journal, 19: 233–268. The second paragraph on page 233 does not mention the writer's preliminary publication (2) on the subject. The fact that Sideris'

Where most of the roots of a plant are infected and have reached the stage just described, the above-ground symptoms are distinctly those of starvation or drought. These symptoms, etiolation, flaccidness, wilting and dying back from the tip, are not specific for the pink-root disease, as they may occur in other diseases or under environmental conditions unfavorable to the host. Likewise the nipple-shape basal plate, mentioned as a symptom by Taubenhaus (10), is not specific for pink root, as it is not always found on infected onions and frequently is found on noninfected ones. In cases of severe infection, the general effect on the host is that of stunting the bulbs which are below a marketable size.

Microscopic Symptoms. On microscopic examination, the root tissues are found to be invaded by a fungus mycelium which often goes up into the root bases imbedded in the bulb plate, but it has not been found in the basal plate proper. The pigment which gives to the infected roots their characteristic pink color diffuses out, or is produced, ahead of the fungus filaments, sometimes to a distance of ten to twelve cells. In certain cells, sometimes close together, sometimes far apart, the mycelium accumulates, swells into various shapes, and turns dark in color. This phenomenon is the beginning of pycnidium formation and, though it may be observed in all parts of the root, it is seen more frequently in the epidermal cells.

The writer engaged in the pink-root problem with the object of determining how many and what species, varieties, or strains of Fusarium are able to produce the disease and which of these, singly or in combination, are most virulent. At the beginning of this work (Dec., 1925) no fresh material was available. Consequently, the preliminary isolations were made from the root remains and bulb plates of dry onions which had been grown in a pink-root infected field at University Farm, Davis, California. These onions still showed unmistakable evidence of having been affected with pink root. For isolation purposes material of this kind is quite undesirable because it absorbs any liquid disinfectant used and surface sterilization of the tissues can therefore not be practiced. Hence many organisms other than the pathogens are invariably obtained. The following method of isolation was used: Small pieces of root and bulb plates were washed with sterile water and placed on nutrient agar in Petri dishes, four pieces in each of twenty plates, giving eighty plantings. From the edges of the resultant colonies more than three hundred transfers were made, yielding many species of Fusarium and other members of the soil flora. From the

work was done prior to 1924 and not prepared for publication until several years later probably explains this omission. At the top of page 235 is listed a number of organisms which were isolated, among these a Phoma. The writer was unable to give credit in his article for this find, since Phoma is not mentioned in the thesis deposited in the University of California library.

Fusarium cultures more than two hundred single-spore isolations were made by the capillary-tube method (1). From these isolations there were obtained the following species of Fusarium which were identified by the usual method of parallel cultures on various media: *F. malli*, *F. cromyopthoron* Sid., *F. rhizochromatistes* Sid., *F. moniliforme* Sheld., and ten additional species, varieties, and strains of the same genus. The latter were not specifically identified but for inoculation purposes labelled F. 1 to F. 10. The isolated fungi were used in the following experiment:

Two hundred and thirty two-inch pots were filled with rich greenhouse soil and steamed for four hours at twenty pounds pressure. Three days after steaming, the soil from five of the pots was cultured on nutrient agar and found sterile; the remaining 225 pots were then inoculated with the isolated fungi as shown in table 1. The inoculum was taken from twenty-day-old cultures on potato slants and worked well into the soil with sterile spatulas. In order to give the fungi a good start the pots were left five days longer before each was planted with three onion seeds of the Yellow Globe Danvers variety. The seeds were treated for 10 minutes with mercuric chloride 1:500 and rinsed with sterile water. The experiment was carried on in the greenhouse at temperatures varying between 25 and 28° C. From time to time after germination plants were removed from the pots and examined for symptoms of pink root. The experiment was terminated forty days after germination when all the seedlings were removed and examined. At that time all the remaining seedlings had well-developed

TABLE 1.—*Results of growing onion seedlings in Fusarium-inoculated soil*

No.	No. pots	No. seedlings	Inoculum	Pink root
1	15	38	*F. malli*	none
2	15	42	*F. cromyopthoron*	"
3	15	42	*F. rhizochromatistes*	"
4	15	40	*F. moniliforme*	"
5	15	37	F. 1	"
6	15	36	F. 2	"
7	15	39	F. 3	"
8	15	33	F. 4	"
9	15	42	F. 5	"
10	15	42	F. 6	"
11	15	45	F. 7	"
12	15	36	F. 8	"
13	15	39	F. 9	"
14	15	39	F. 10	"
15	15	38	none (controls)	
Totals	225	588		None

root systems but were somewhat crowded in the small pots. The results of the experiment are given in table 1. Seeds planted December 19, 1925. Experiment terminated February 10, 1926.

As shown in table 1, the results were entirely negative; no pink root or other abnormalities of the roots were observed. In view of the positive results obtained by Taubenhaus (10) and by Sideris (6) it seems probable that in my work the organisms to which the disease was attributed had not been used. Hence supposedly authentic cultures of *F. malli* and *F. cromyopthoron* were obtained (the culture of *F. malli* was one Sideris had been working with at Berkeley and the culture of *F. cromyopthoron* was sent sent to me by him from Hawaii), and the inoculation experiment repeated on 24 plants; 6 inoculated with *F. malli,* 6 with *F. cromyopthoron,* 6 with both, and 6 left uninoculated as controls. Both Sideris and Taubenhaus agree that high temperatures and other conditions unfavorable to the host favor the development of pink root. During the present experiment temperatures were kept between 28° and 32° C., water was applied in varying amounts at irregular intervals, and the root systems of the inoculated plants were mutilated by cutting down through the soil with a knife. Two months later there was no evidence of pink root or other disease symptoms, indicating that either some important factor had been overlooked or that the supposedly authentic cultures of Fusarium were not pathogenic to onions. At this time (May, 1926), work with the fungi isolated from dry onions was discontinued, new isolations were made from infected roots of young, growing onions obtained from the following places: Stockton, Sacramento, the Imperial Valley, and University Farm, Davis. Culturing the fresh material gave results somewhat at variance with those previously obtained from dry onions, a result no doubt due to the fact that surface sterilization could be practiced on this material. The new isolations were made by immersing pieces of infected roots in mercuric chloride 1:500 for three minutes, rinsing in sterile water to remove excess fungicide, and culturing on Czapek's nutrient agar. Roots in advanced stages of the disease yielded a few species of Fusarium and a species of Phoma. Roots in the very early stages, when still turgid and firm, though distinctly pink, yielded no Fusaria but only a species of Phoma. The fact that the latter organism occurred on diseased material from all of the above-named places indicated that it might be an important factor in the pink-root disease.

In view of the results obtained by myself and previous investigators special precautions were taken to have the pathogenicity test of this new fungus conducted under as nearly aseptic conditions as possible. The bottom of each of ten 500 c.c. Erlenmeyer flasks was covered with about four centimeters of clean sand wetted with nutrient solution. The flasks

were stoppered with cotton plugs and autoclaved for three hours at twenty pounds pressure. After they had cooled, eight of the flasks were inoculated with *Phoma* sp. and two left uninoculated as controls. The inoculum was taken from twenty-day-old cultures on Czapek's agar and consisted of about ten grams of mycelium and medium. Each of the ten flasks was then planted with four onion seeds (Yellow Globe Danvers) which had been immersed in mercuric chloride 1:500 for fifteen minutes. Ten days after germination, many of the seedlings in the inoculated flasks showed typical symptoms of pink root and in four weeks all were dead. The controls remained healthy. Upon culturing the onion remains and sand from the inoculated flasks the fungus was obtained in pure culture. Culturing the seedlings and sand from the controls gave no cryptogamic flora whatever. The above experiment was repeated with rooted onion bulbs as follows: 24 onion bulbs (Ebenezer variety) were immersed in a 0.5 per cent solution of Semesan for 30 minutes, and placed on bottles containing a nutrient solution. When the roots were three inches or more in length the cultures were inoculated as follows: 6 with *Phoma* sp., 6 with *Fusarium malli,* 6 with *Fusarium cromyopthoron,* and 6 left uninoculated as controls. The Fusarium inocula were from three different sources, viz: 1, isolated from root remains of dry onions; 2, isolated from fresh onion roots, and 3, ob-

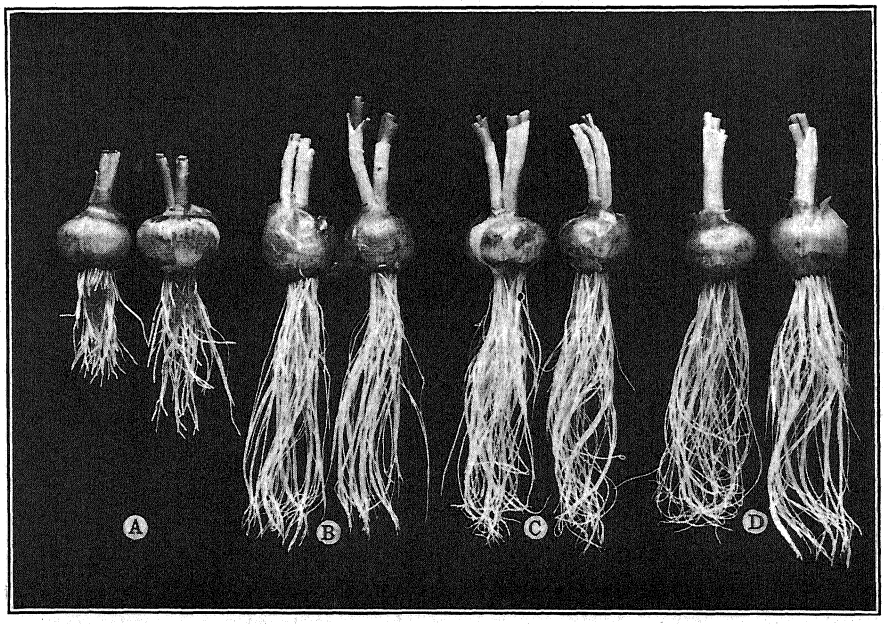


FIG. 1. A. Inoculated with *Phoma* sp. B. Inoculated with *Fusarium cromyopthoron.* C. Inoculated with *Fusarium malli.* D. Controls.

tained from Sideris. Each inoculum, consisting of about five grams of fungus and medium, was floated on the surface of the nutrient solution in contact with the roots. The experiment was initiated May 28, 1926, and terminated July 18, 1926. The results are given in table 2 and illustrated in figure 1.

TABLE 2.—*Comparative parasitism on onions of Phoma sp. and Fusarium spp.*

Culture No.	Inoculum	Pink Root
1– 6	*Phoma* sp.	all infected
7– 8	*Fusarium malli* No. 1	none "
9–10	" " " 2	" "
11–12	" " " 3	" "
13–14	*F. cromyopthoron* No. 1	" "
15–16	" " " 2	" "
17–18	" " " 3	" "
19–24	Control	" "

The results of the previous experiments indicate that the cause of pink root in California is *Phoma* sp. rather than *Fusarium malli, Fusarium cromyopthoron,* or any other species of that genus. It would seem very likely, however, that species of Fusarium might act as secondary parasites and thus contribute to the virulency of the disease. The following experiment was devised to test this possibility: 240 half-pint milk bottles were filled with washed sand, wetted with a nutrient solution, and autoclaved for four hours at twenty pounds pressure. The bottles were then inoculated as follows: 100 inoculated with *Phoma* sp. plus *Fusarium* spp. (consisting of a mixture of spores from more than fifty cultures of *Fusarium* comprising the isolations made earlier from both dry and fresh material and from identified cultures of *F. malli, F. cromyopthoron,* and *F. rhizochromatistes*). The inocula were applied as spore suspensions in sterile tap-water. Forty bottles were left uninoculated as controls. After inoculation an onion bulb (Ebenezer variety), treated for 30 minutes with 0.5 per cent solution of Semesan, was placed on each bottle. For the first six weeks of the experiment no difference could be detected between the onions inoculated with *Phoma* sp. alone and those inoculated with both *Phoma* sp. and *Fusarium* spp. After that time, however, the latter proceeded to go down more rapidly and at the time the experiment was concluded (90 days after inoculation) nearly all were dead, while those inoculated with *Phoma* sp. only were still alive (Fig. 2). Upon microscopic examination it was found that in the onions inoculated with both *Phoma* sp. and *Fusarium* spp. the bulb plates and, in most cases, the bulbs themselves were heavily infected with mycelium of Fusarium. Where *Phoma* sp. alone had been used as

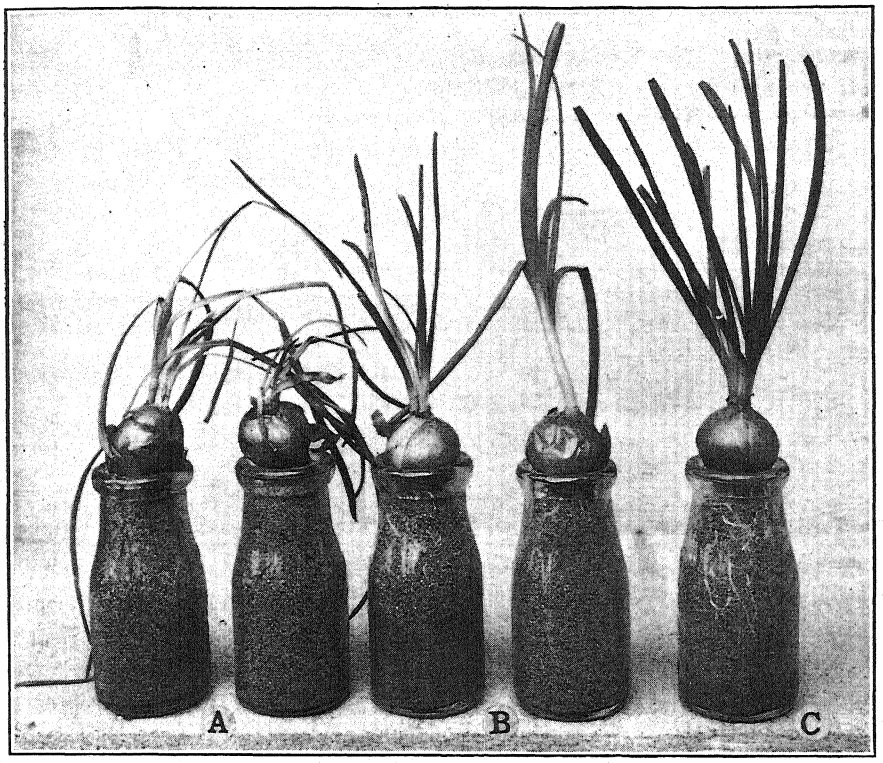


FIG. 2. A. Inoculated with *Phoma* sp. and *Fusarium* spp. B. Inoculated with *Phoma* sp. C. Control.

inoculum the bulbs and bulb plates were not invaded. Results of the above experiment indicate that where the host has already been invaded by a parasite, species of Fusarium are able to enter and materially aggravate the diseased conditions.

TEMPERATURE RELATIONS

The following experiment was designed to determine the optimum temperature for infection of onion roots by *Phoma* sp. Twenty-four shallow flower pots, 6 cm. deep by 30 cm. in diameter, were filled with loam soil and autoclaved at 20 pounds pressure for four hours on three successive days. Each of these pots was heavily inoculated with cultures of *Phoma* sp. and planted with five Ebenezer onion bulbs which had been immersed in a 0.5 per cent solution of Semesan for 30 minutes. Four pots were placed in each of six constant temperature chambers. As none of these chambers were equipped for illumination it was necessary to use onion bulbs rather than seedlings. Experimenting with plants grown from bulbs has the additional advantage that, in them, roots and tops can develop for a con-

siderable length of time independent of outside sources of energy or food materials, and also to a certain extent independent of one another; hence, infected roots would not be expected materially to affect growth of the tops and, therefore, temperature effect on growth of the host and on degree of infection could be studied in the same plants. Results of the experiment are given in table 3.

TABLE 3.—*Effect of temperature on pink-root development*

No.	Temperature	No. of bulbs	Plant development[1]	No. of plants infected	Per cent infected
1	4 C.	20	10	0	0.0
2	10 C.	20	30	0	0.0
3	13 C.	20	40	1	5.0
4	20 C.	20	80	15	75.0
5	25 C.	20	100	20	100.0
6	30 C.	20	70	4	25.0

[1] Rather than classify plant development as very poor, poor, fair, etc., a numerical classification was adopted by arbitrarily giving the lot of plants with greatest growth the rating of 100 and measuring the others by this standard.

It will be seen from the foregoing results that apparently no infection takes place at 4° and 10° C. and but little at 13° C. These temperatures, however, are far too low for normal development of the host. As indicated in table 3, the highest percentage of infection coincides with the greatest growth of the plants.

TABLE 4.—*Growth of Phoma sp. at various temperatures*

Temperature in degrees C.	4 days	8 days	12 days	16 days	20 days
	Diameter of colonies in mm. at indicated intervals				
4	0	0	0	0	0
10	0	0	0	0	0
13	0	3	5	8	12
20	10	26	42	59	76
24	20	38	56	76	90[1]
25	21	40	59	78	90
26	22	42	63	84	90
28	20	37	56	75	90
30	17	34	51	70	85
32	9	20	32	44	56

[1] Plate overgrown.

In order to correlate the effect of temperature on infection and on maximum growth of the fungus, fifty plates, each containing 10 c.c. of corn-meal agar, were inoculated with spores of *Phoma* sp. and incubated at constant temperatures. Five plates were placed at each temperature and the colonies measured at four-day intervals. The measurements given in table 4 are averages of five colonies.

It will be observed from the above results that *Phoma* sp. grows very slowly at the lower temperatures and that the optimum is not far from 26° C. Comparing the results in tables 3 and 4, we find that the optimum temperature for infection of onion roots is also optimum for best growth of *Phoma* sp. in culture and perhaps also optimum for host development.

THE PATHOGEN

In reading descriptions of the thousands of species of Phoma in Saccardo's Sylloge Fungorum (4) and in other mycological works, one can not fail to notice how closely many of the species resemble one another morphologically. Seemingly habitat or host has in many instances served as the only basis of separation. It might, therefore, seem unwise to encumber the literature further by adding a new species to a genus already so numerously represented. However, in view of the fact that the newly isolated fungus differs considerably morphologically and culturally from the comparatively few other species of that genus described as root parasites, I think that its recognition is justifiable and suggest for it the name **Phoma terrestris,** n. sp.

Type Morphology. Pycnidia subglobose, ostiolate, papillate, dark brown to black, carbonaceous, 170 to 350 μ, may occur singly, frequently gregarious (Fig. 4). Conidia continuous, hyaline, oblong-ovoid, 4.5–5.5 × 1.8–2.3 μ biguttulate, sessile in pycnidium, escaping as a gelatinous mass through ruptures rarely as a gelatinous cirrus through the ostiole. Mycelium septate, hyaline, guttulate 1.0–4.5, frequently anastomosing.

On Czapek's, Conn's, Board of Health, Pfeffer's, and other synthetic media of the same type, there is very little pycnidium formation and, as a study of the physiology of the fungus was not intended, they were little used. The colors produced in these media are essentially the same as those occurring in invaded onion roots with the exception that in very old cultures, 3 months or more, the color often becomes nearly black, due to darkening of the mycelium. Corn-meal agar was found to be well suited to the formation of both pycnidium and pigment. On this medium the color varies between deep rose pink to rose lane purple in old cultures. If spore transfers are made to this medium, pycnidia usually are formed in from 3 to 5 days; if transfers are made from mycelium, pycnidia usually do not begin to form for from 10 days to 3 weeks. The capacity of *Phoma terrestris* to form pycnidia becomes markedly greater by making transfers from spores only and also

markedly less if mycelial transfers are repeatedly made (Fig. 4). One of my cultures (Fig. 4, A), derived from repeated mycelial transfers, has been grown on corn-meal agar for 60 days without producing pycnidia. This is not a phenomenon peculiar to Phoma, as Wineland (12) and several other investigators have observed similar behavior in species of Fusarium. Pycnidium formation apparently begins by swelling and lateral division of hyphal cells, giving rise to conglomerate masses of dark thick-wall bodies somewhat resembling chlamydospores (Fig. 3). These bodies are always

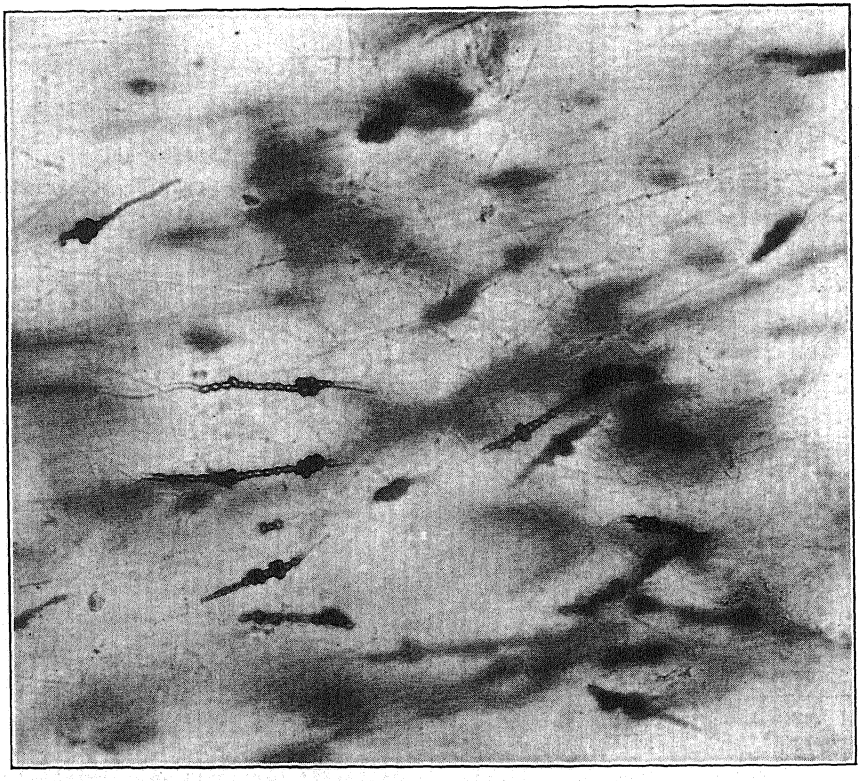


FIG. 3. *Phoma terrestris*, n. sp. Beginning of pycnidium formation in corn-meal agar. Similar bodies are formed within the host cells.

found in the cells of infected onion roots, where mature pycnidia apparently never occur. Here the bodies reach about the same stage of development obtained in media containing comparatively large amounts of free sugars, which seem to be a deterrent to the formation of mature pycnidia. It is likely that they serve as a carry-over or resting stage of the fungus. The mature pycnidia vary considerably not only in size, as indicated in the technical description, but also in shape, papillation, and in length and number

of the ostioles. The cultural differences of the various isolations shown in figures 4 and 5 might be deemed of varietal magnitude. Considering, however, the derivation of A and B from C of figure 4, and, further, considering the fact that all the variants are able individually and collectively to cause the pink-root disease of onions, it is the writer's opinion that all the variants should be classified as strains of *Phoma terrestris.*

Distribution. As previously stated, *Phoma terrestris* has been found on onion roots from various parts of the State and the writer maintains that it, or strains of it, constitute the cause of pink root of onions everywhere. Shortly after my first publication in 1926 (2), E. L. Felix, of the Plant Pathology Division at Cornell University, sent me a culture of a fungus

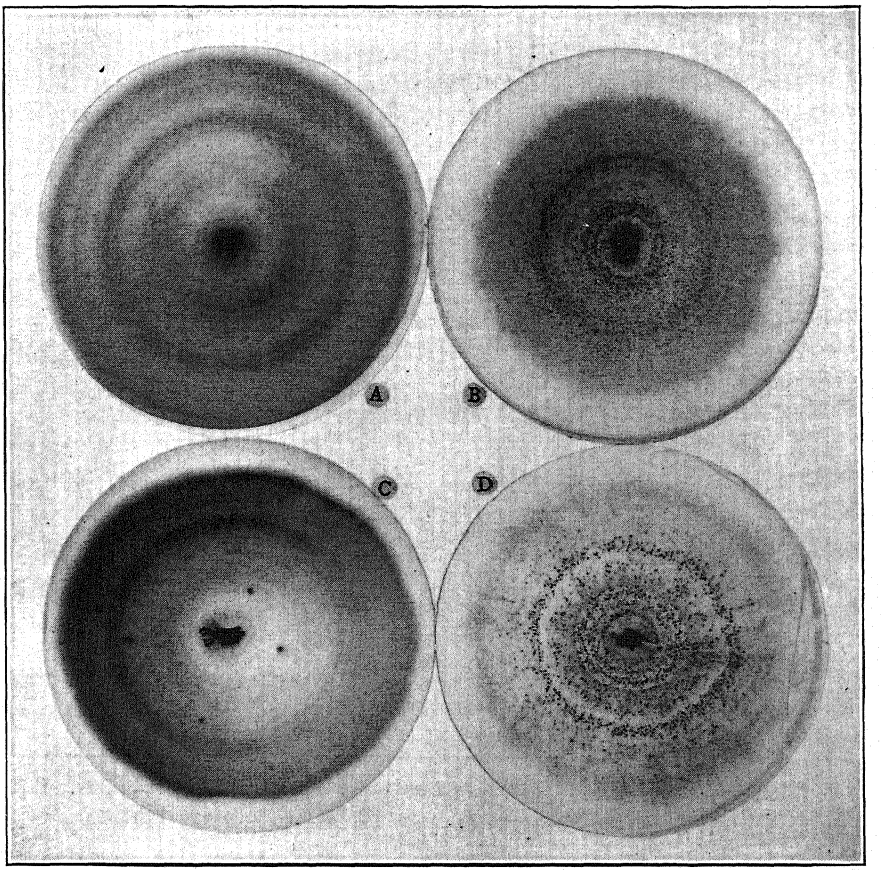


FIG. 4. *Phoma terrestris,* n. sp. A. Derived from C by constant mycelial transfers. B. Derived from C by constant spore transfers. C. Original isolation from diseased onion roots (California). D. Isolated from diseased onion roots (Wisconsin).

with which he had been able to produce pink root of onions. This organism appeared identical with mine except that it did not produce mature pycnidia; later, when Mr. Felix was in Bermuda, he graciously sent me some preserved material from there and, though this material could not be cultured, microscopic examination revealed the presence of mycelium similar to that of *Phoma terrestris* or a closely related species. In 1927, I received several cultures of Phoma from L. F. Alexander, stationed at Madison, Wisconsin. In a letter Mr. Alexander states that he invariably failed to pro-

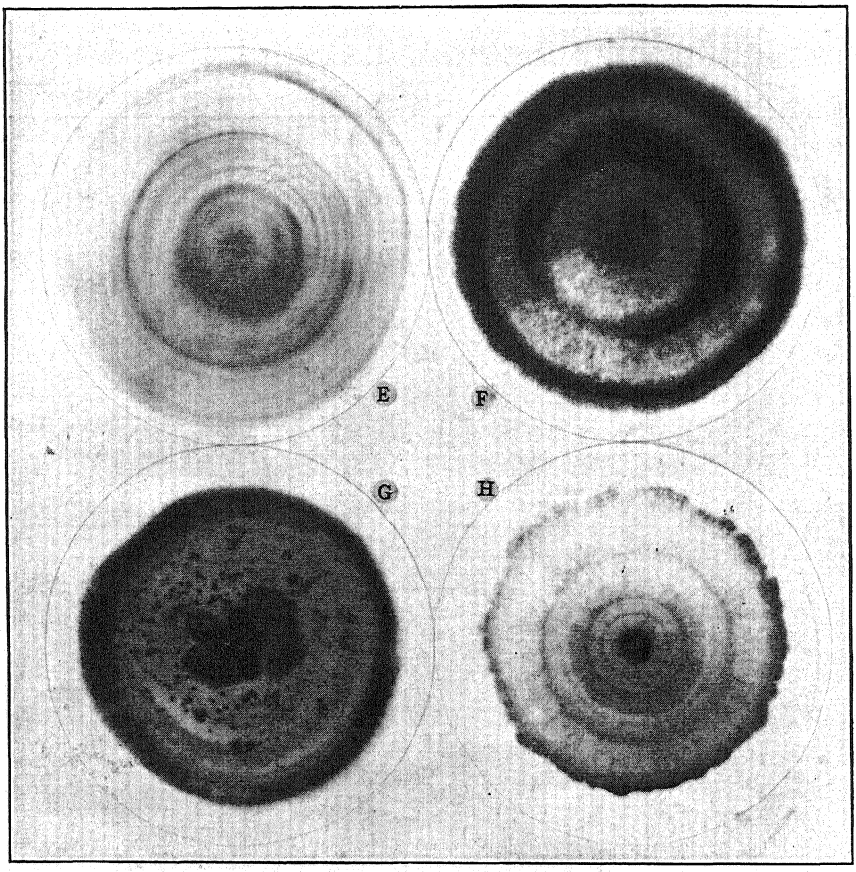


FIG. 5. *Phoma terrestris,* n. sp. E. Isolated from potato seed piece (California). F and G. Isolated from diseased onion roots (Texas). H. Isolated from diseased onion roots (New York).

duce pink root of onions with species of Fusarium but that he always obtained positive results when inoculating with the Phoma-like fungi. Of the cultures sent by Mr. Alexander, one was identical with that obtained from

Felix, one apparently identical with mine, and one differing slightly in abundance of pycnidium formation.

In 1928, I isolated two strains of *Phoma terrestris* from pink-root-infected onions grown in Bexar County, Texas (Fig. 5, F and G). I have also isolated the fungus from lesions on the roots of cowpea, *Vigna sinensis* Endl., lima bean, *Phaseolus limensis* Macf., and from potato seed pieces. The importance of the fungus as a pathogen on these and other hosts is being further studied. The above indicates that *Phoma terrestris* is not confined to members of the genus Allium but that it is a common soil habitant of wide distribution, capable of attacking the roots of quite divergent genera of plants.

SUMMARY

1. The disease known as pink root of onions has been further studied and described. The work of previous investigators is briefly discussed. They conclude that the disease is caused by species of Fusarium.

2. Inoculation experiments in sand, soil, and water cultures under various conditions with *Fusarium malli*, *F. cromyopthoron*, *F. rhizochromatistes*, and ten other species, strains, and varieties of Fusarium gave negative results, indicating that, as far as California is concerned, members of the genus Fusarium are not responsible for the pink-root disease.

3. A fungus belonging in the genus Phoma was isolated from living onion roots affected with the pink-root disease. Inoculation experiments with this fungus under controlled conditions invariably gave positive results, indicating that it is the true causal agent. The new fungus is described and the name **Phoma terrestris**, n. sp., suggested.

4. Inoculation experiments with both *Phoma terrestris* and *Fusarium* spp., seem to show that species of Fusarium act as secondary parasites and hasten destruction of the host.

5. Evidence is presented that *Phoma terrestris* is the cause of pink root of onions, not only in California but in other States as well, and that hosts other than Allium may be attacked.

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LENTINUS LEPIDEUS FR.: A CAUSE OF HEART ROT OF LIVING PINES

W. W. WAGENER

The scaly Lentinus, *Lentinus lepideus* Fr., is well-known as a saprophyte, decaying coniferous stumps, logs, foundation timbers, ties, etc., over a wide range in the North Temperate Zone.

As a heart rot of living trees it appears to be much less familiar. Hedgcock (3) in 1912 reported it or a closely related form as attacking the heartwood of both living and dead conifers in the National Forests but, owing to the scope of the paper, no details of his observations were included. Meinecke (6), in speaking of the sporophores of the fungus found on the butts of *Pinus jeffreyi* A. Murr., *P. ponderosa* Doug., and *P. contorta* Doug. in California, stated that occasionally they grew "on the dead roots of living trees and also high up on the trunk." A species of Lentinus parasitic on the roots of conifers in the western United States has been mentioned by Weir (8). He regarded it as a probably undescribed species but has verbally informed the writer that the fungus to which he referred is the one commonly known as *Lentinus lepideus* in the West. More recently, Martin (5, p. 407) observed sporophores of the fungus growing from branch scars on a living pine in Washington, D. C. While the occurrence of *Lentinus lepideus* or closely related forms in connection with living trees has thus been reported for some time, the fact seems not to have gained general attention and is not referred to in our pathological texts.

The situation is no doubt due in part to the uncertainty, as voiced by Hedgcock and Weir, regarding the true identity of the western fungus. While there appears to be some difference between the western form and the common one of the eastern United States and Europe, yet, as Harper (2) has pointed out, the species is everywhere quite variable and it is questionable whether the western fungus is any more entitled to specific rank than any one of the morphological variations found elsewhere. In the present paper common custom will be followed in referring to the fungus simply as *Lentinus lepideus* without entering the question as to the correctness of such usage.

The original report by Hedgcock was based mainly on field notes and collections made by him in connection with the Timber and Forest Disease Survey conducted for the United States Department of Agriculture some years ago. The fungus was noted at several places in the Western and Middle-western States with living *Pinus ponderosa, P. contorta,* and *P. banksiana* Lamb. as hosts. In some instances sporophores were found con-

nected with a brown heart rot within the tree, from which the fruiting bodies issued through knotholes or open wounds. In general he found that the rot appeared to be confined to the heartwood but in one lodgepole pine (*P. contorta*) the sapwood adjacent to a basal fire scar was also involved.

Collection and field notes in the files of the San Francisco Office of Forest Pathology contain a number of records of the fungus observed on living western yellow pine (*P. ponderosa*) and lodgepole pine by Meinecke. Sporophores were noted growing from the bases of trees, from roots near the base or directly from decayed heartwood through openings in the trunks.

FIELD OBSERVATIONS ON INDIVIDUAL CASES IN CALIFORNIA

Within the past few years additional finds of the fungus have been made in California on living western yellow pine and on sugar pine (*P. lambertiana* Doug.), a previously unrecorded host. Several of these cases were

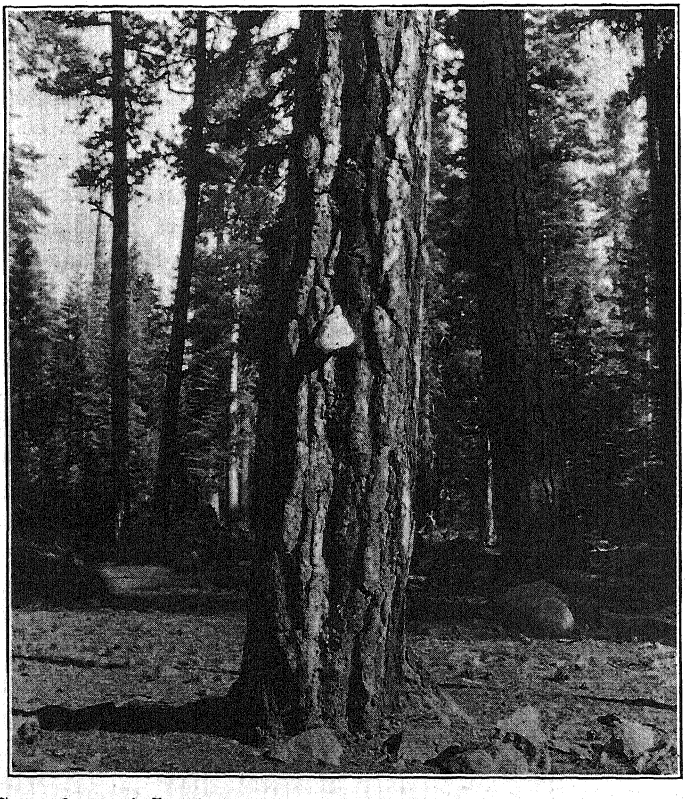


FIG. 1. Sporophore of *Lentinus lepideus* growing from an old, bark-closed wound on *Pinus ponderosa*, Westwood, California.

encountered in the course of a study of decay carried out under the direction of Dr. E. P. Meinecke in connection with the logging of a government timber sale in the central Sierra Nevada Mountains. The stand was of mixed conifers of large size, mostly mature, growing at an elevation of 5000 feet.

The first case noted occurred in a western yellow pine measuring 56 inches in diameter breast high and originally 180 feet tall. At the time of felling, early in September, only a standing snag 70 feet in height remained, the entire top having broken out during a severe windstorm the previous fall. The diameter of the bole at the break was about 28 inches. Western yellow pine is ordinarily very resistant to breakage of this sort and the fact that no similar damage was shown by adjacent trees pointed to some weakness of the bole in the region of the break.

The tree was examined about four weeks after felling. A dry, mature sporophore of *Lentinus lepideus* about 15 cm. in diameter was found projecting from a recess in the decayed wood on the top end of the snag. It arose from a felty, mycelial pad formed over the surface of the wood and was of the normal pileate type. Its shape and condition indicated that the sporophore had matured and hardened on top of the snag before felling.

The lower end of the long broken-off top which had lain on the ground over winter bore a sterile fructification consisting of a curving, branched, compound stipe with brown, conically pointed tips. It arose from a mycelial pad similar to that at the base of the normal sporophore, except for soil and litter embodied in the surface. The production of sterile fructifications of this type is known to be a common phenomenon for *Lentinus lepideus*. The shapes are frequently grotesque (7, p. 411). Experiments by Buller (1) showed that light has a strong influence in determining the form of the fruiting body. In the present case the normal sporophore developed in full sunlight while the end of the break bearing the abortive one, lay so that it was constantly, though not heavily, shaded. In view of Buller's experiments, this may account for the dissimilar development of the two fruiting bodies. Normal pileate sporophores were later produced from both portions of the down tree.

Decay and discoloration in connection with the sporophores extended roughly spindle-shape for 12 feet above and 8 feet below the break, or a total distance of 20 feet along the bole in both heartwood and sapwood. The rest of the tree was sound. The decay centered around a long, narrow wound, dating from 1895, on the side of the bole and probably due to a falling tree or snag. This wound quite evidently served as the entrance point for the fungus, its subsequent development weakening the bole so that it was unable to withstand the stress of the heavy wind.

An important factor in the weakening was the death of the callus-tissue and sapwood for about a foot on each side of the wound after normal callus formation had continued for a number of years. The dead area was found to be in various stages of decay from Lentinus and was covered for the greater part by a creamy-white mycelial felt about 1/16 of an inch thick, which had formed between the wood and the overlying bark. No definite cause could be ascribed for the death of this zone of sapwood. However, the presence of the fungus apparently throughout the decayed area and the absence of indications of any other cause for death suggested its possible connection with the killing.

The rot itself was not pronounced except in the vicinity of the wound where, for a depth of about 6 inches, the wood had disintegrated into long, rectangular, brown flakes or chunks of carbonaceous texture. The rest of the affected wood appeared solid in cross section, but, when tested with the point of a knife blade, proved to be softer than normal and lacking in elasticity. Thin mycelial felts showed in longitudinal section but were confined to the small shrinkage checks near the surface of the break. The color of the wood was a slightly darker yellow than normal, shading into a reddish tinge in places. The sporophores did not develop from the badly disintegrated outer wood but from the more solid heartwood about 10 inches in from the outside of the trunk.

A loss of 3000 board feet resulted from the decay and the breakage caused by it in this tree.

A second case of the rot was disclosed by the felling of a living yellow pine 40 inches in diameter and 188 years of age at stump height. The entire heartwood at the stump was involved in a spongy, reddish brown decay with a resinous, aromatic odor suggesting *Lentinus lepideus*. A cluster of two sporophores and an abortive stipe of the fungus were found hidden under a layer of needles and dry duff about 2 feet from the base of the tree. They arose from a shallow, decayed root about an inch in diameter. The decay appeared to connect through this root with that in the butt of the tree though conditions prevented excavation to determine this point definitely. Some support was afforded by the fact that agar cultures of the decayed wood from the stump gave typical *Lentinus lepideus* growths. The rot was confined to the butt of the tree, resulting in a deduction for cull of 140 board feet in the scale of the first log. Infection had presumably taken place through a fire scar, open since about 1850, at the base of the tree.

A third case of the disease was found in the upper bole of a sugar pine 67 inches in diameter breast high. The tree had been broken off at 133 feet from the ground, resulting in the loss of much of the crown but leaving a number of large live limbs just below the break. The diameter at the break

was about 14 inches, but it increased rapidly below this point. Decay extended down in the heartwood of the trunk for 11.5 feet, attaining a maximum diameter of 20 inches. It also entered the bases of several of the live branches. The center for some distance down had become hollow, and attached to the decay on the sides of the hollow were a number of stained and misshapen fruit bodies, mostly abortive, and some so covered with bits of decayed wood and débris as to be scarcely recognizable. One or two had developed sufficiently to be identified as *Lentinus lepideus*. The length of time the fungus had been present could not be determined. A cull loss of about 100 board feet resulted.

Several additional living yellow pines affected with the decay have since been noted by the writer. In one of 45 inches diameter, found later on the same sale area, the fungus had entered through an old broken top and had destroyed the heartwood for 40 feet below, resulting in a cull loss of 1060 board feet. The decayed portion of the bole split its entire length when the tree was felled.

Another tree, 48 inches in diameter, on which a Lentinus sporophore had been seen issuing from an exposed root, was examined. Some years before, a road had been excavated on the slope immediately below this tree, resulting in the soil and a number of large roots being cut away on the lower side. Other roots were partly exposed. Due to the location it was impossible to fell the tree for examination but ax cuts and soundings showed the base to be badly decayed for at least 8 feet above ground. On the side next to the road the sapwood was dead and had decayed until only a thin shell of firmer dry wood remained on the surface.

About a month intervened between the time the sporophore was noted and the time the examination was made. In the meantime the fruiting body had been removed by man or animal so completely that not even its point of attachment could be definitely determined.

The root from which it was thought to have issued was about 4 inches in diameter and was virtually exposed for about a foot of its length, beginning three feet out from the tree. A cut made through at the middle of the exposed length showed nearly two-thirds of the cross section decayed. The rest was still alive. The root was next excavated back to its junction with the base of the tree and opened up. It was seen that the decay continued back and connected with the main body of rot in the base of the trunk.

In order to make certain that the decay in the root and that in the trunk were identical, agar cultures were made of each. The growths obtained agreed in all particulars with each other and with authentic cultures of the fungus. This case supports previous indications that the decay may extend into the roots of a tree while the latter is still alive.

THE DECAY

Early stages of the decay in living pines have no very marked characteristics. The wood becomes brash and darkens slightly in color, with a faint reddish tinge appearing in some cases. Little or no shrinkage in excess of that in the normal wood takes place. Thin mycelial felts are formed in checks or shakes, particularly in portions from which sporophores will later develop.

In later stages the decayed wood is friable, crushing to a powder when rolled between the thumb and finger, but with a cheesy or sticky character when fresh due to contained moisture and to infiltration of oleoresins. These latter the fungus appears to be able to liberate in unusual degree. The infiltration is particularly noticeable in decay in the butts of trees or in the vicinity of fire scars or wounds and causes the decay to appear darker in color than when dried out.

The essential oils volatilize rather rapidly from freshly-exposed decay, giving it a highly aromatic, resiny odor, noticeable at some feet distant. A fragment no larger than one's little finger will, when freshly broken, sometimes scent an entire room. Coupled with the resiny odor is one of a fungous nature, the combination making an odor peculiar, so far as the writer knows, to the decay of *Lentinus lepideus* and readily recognized, once one has become acquainted with it.

Some shrinkage cracks form in fresh decay of the later stages but, as a rule, it is not until the decay has been exposed and subjected to drying that it breaks up into the brittle, cubical chunks typical of the carbonizing rots. In *Lentinus lepideus* the chunks are rather large and rectangular, corresponding in size to those of *Fomes laricis* (Jacq.) Murr. except in sapwood where smaller cubes are formed. A variation sometimes found in decayed heartwood is the breaking up into thin rectangular flakes, the wood separating tangentially along the annual rings. This is brought about by the greater resistance of the summerwood to the action of the fungus and is more likely to be found in intermediate stages of the decay. In color, dry decay varies from cinnamon buff and clay to cinnamon and sayal brown, weathering on exposure to a dark brown or grayish brown. Thin, tough felts of mycelium are ordinarily present.

The dry decay sometimes resembles that of *Fomes laricis* so closely that specimens of each placed side by side can not be told apart visually though a distinction can easily be made on the basis of odor. The microscopic characters of the two decays are very similar (4). Old weathered decay is almost indistinguishable from that of *Polyporus schweinitzii* Fr. In a fresh state the decay, because of its odor, absence of checking into cubical form, and its consistency, can scarcely be confused with either of the others mentioned.

As indicated by the field cases reported, the rot is not characteristically limited to any particular portion of the bole. However, owing to the concentration around the base of the tree of wounds favorable to its entrance, infections of the fungus are more likely to be found in the heartwood of the butt.

HOSTS AND DISTRIBUTION

A summary from records at hand shows the following distribution for observed cases of the fungus on living trees:

California	on	*Pinus*	*ponderosa*	7	localities.
"	"	"	*contorta*	2	"
"	"	"	*lambertiana*	1	locality.
Arizona	"	"	*ponderosa*	1	"
Montana	"	"	*contorta*	1	"
Minnesota	"	"	*banksiana*	1	"
Dist. of Columbia	"	"	sp.	1	"

For several of the Californian localities and also for the one in Arizona more than one case of the rot has been found.

These distributional records and supporting field observations indicate that decay from *Lentinus lepideus* in living pines is not uncommon in the west Sierran region of California but to be found only occasionally or rarely elsewhere in the country. The Californian localities are well distributed over the west slope of the Sierra Nevada range.

The reasons for the apparent greater prevalence in California are not entirely clear. It may be noted in this connection however that the fungus is an extremely common saprophyte on stumps and down logs in the region and that the yellow-pine stands have a heavy representation of large, mature trees, most of which bear fire scars or wounds of some sort.

SUMMARY

Lentinus lepideus, well-known as a saprophyte, also causes a heart rot of living pines, particularly of the western yellow pine, *P. ponderosa.* It also has been found in *Pinus contorta, P. lambertiana,* and *P. banksiana.*

The fungus enters through wounds and may attack any part of the heartwood of the bole or extend into the roots. In rare cases the sapwood is also affected.

A friable decay, often characterized by an infiltration of oleoresins, is caused. The dry decay resembles that of *Fomes laricis* but may be distinguished by the resiny, fungous odor.

Field observations and collection records indicate that the decay is fairly common in living pines in the forests of the Sierra Nevada in California and rare elsewhere in the United States.

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THE USE OF ACIDULATED MERCURIC CHLORIDE IN DISINFECTING POTATO TUBERS FOR THE CONTROL OF RHIZOCTONIA[1]

J. G. LEACH, H. W. JOHNSON, AND H. E. PARSON[2]

Experimentation for many years by numerous workers throughout the United States has demonstrated conclusively that seed-tuber disinfection is an effective means of reducing losses from scab, blackleg, and rhizoctonosis. Tuber disinfection has never given perfect control of these diseases because none of them is exclusively tuber-borne. Nevertheless, under most conditions, thorough tuber disinfection has greatly reduced the amount of infection.

Since the early work of Bolley (2) and Arthur (1) many different disinfectants have been used and many different methods of application have been recommended. Some of these have been very effective, while others have been less valuable. Perhaps the most successful disinfectants have been formaldehyde and mercuric chloride. These have proved very effective in countless experiments, and for many years have been used as standards of comparison.

In determining the value of a method of tuber disinfection there are two factors of major importance to consider: First, the effectiveness of the treatment in disinfecting the tubers, and second, the practicability of the method in the hands of the farmer.

The lethal action of a disinfectant on the sclerotia of Rhizoctonia generally has been considered as the limiting factor in determining its effectiveness, for any disinfectant which penetrates and kills the sclerotia doubtless would destroy the pathogenes causing blackleg and scab.

In judging the practicability of the method in the hands of the farmers one must consider such factors as the probability of injury to the seed tubers, the cost of materials, the availability of necessary facilities for proper application, and the time and labor involved. Experience has shown that unless these factors are favorable the method is not likely to be used generally, regardless of its merits as an effective disinfectant.

The principal objections of potato growers to the use of tuber disinfection have been the time, trouble, and labor involved. For many years, the long-soak formaldehyde and mercuric-chloride methods were the stand-

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[2] The authors wish to express their appreciation to Mr. T. M. McCall, Horticulturist of the Northwest School and Station, Crookston, for his splendid cooperation throughout this work and for his care of the experimental plots at Crookston.

ard methods recommended by most experiment stations. These methods were practicable when only a few bushels were to be disinfected, but the time, labor, and equipment were almost prohibitive when tubers for large acreages were to be treated.

The first successful effort to remedy these difficulties was made in 1919 by Melhus and Gilman (5) in devising the hot-formaldehyde method. The hot-mercuric-chloride method also has been used successfully in some States. The virtue in the use of hot solutions lies in their more rapid penetration and killing of the Rhizoctonia sclerotia and other tuber-borne pathogenes. It has been found that equally good or better disinfection could be obtained in three minutes at 49° C. than in two hours by the old two-hour soak in the cold solution. The use of hot solutions reduced the time and labor required and has been much more practicable than the old two-hour cold-soak method when relatively large quantities of tubers are involved.

The method has, however, introduced other difficulties, the most important of which are (a) the necessity of more complicated apparatus for heating the solution, (b) the difficulty of accurately controlling the temperature, (c) the danger of injury to the tubers if the solution is allowed to become a few degrees too hot, or (d) incomplete disinfection if the solution is allowed to become too cool.

Realizing these difficulties, an effort has been made to devise a method of disinfection which would eliminate the disadvantages but retain the advantages of the short hot treatment. While planning these experiments the attention of the writers was called to the trials of Cunningham with acidulated mercuric chloride. Cunningham in 1925 (3) found in a series of laboratory experiments that the addition of a small amount of hydrochloric acid to the mercuric chloride solution greatly increased its effectiveness in killing the sclerotia of *Rhizoctonia solani* Kühn. Among other things, he demonstrated that if 1 per cent of hydrochloric acid was added to a 1–1000 solution of mercuric chloride complete killing of the sclerotia resulted when infected tubers were soaked for only five minutes and then covered with wet sacks over night. In the following year, Cunningham and Neill (4) presented data from a field test based on the previously reported laboratory tests. From these data they concluded that the method, "though giving excellent results in the laboratory, failed to control Corticium disease in the field."

Although the data presented did not show consistent increases in yield from disinfected tubers as compared with the controls, they did show a fairly consistent and decided reduction in the number of sclerotia developing on tubers produced by disinfected seed pieces. Inasmuch as the production of uniformly high-grade tubers as well as clean tubers for seed purposes is frequently as important as or more important in the United

States than increased yields, it appeared that the method gave more promise than the conclusions of Cunningham and Neill would indicate.

The acidulated-mercuric-chloride method, in various combinations, has been tested in comparison with other methods for three years in Minnesota. The results obtained appear sufficiently promising to justify its recommendation for more extensive trial. The data for the three years are presented in this paper.

MATERIALS AND METHODS

The trials were made in two different parts of the State. One set of trials was made at University Farm, St. Paul, and the other at the Northwest School and Station at Crookston, Minnesota. In general, the rhizoctonosis is somewhat more prevalent at the latter place and soil infestation is more common.

Tubers of the Early Ohio variety bearing Rhizoctonia sclerotia were used in all experiments. Each year some of the tubers were disinfected before cutting and some after cutting, but, as no appreciable differences were obtained, the results have been considered together. In all trials, except those at University Farm in 1926 and 1927, each plot consisted of 50 seed pieces planted 14 inches apart in rows 3 feet apart. At University Farm, in 1926, the plots consisted of only 24 plants. For data on tuber infection the number of plots for each treatment were as follows:

St. Paul, 1926, three; 1927, five; and 1928, four.

Crookston, 1926, three; 1927, four; and 1928, seven.

The data, therefore, are based on approximately 222 plants for each treatment in 1926, 450 in 1927, and 550 in 1928.

In 1927, the St. Paul plots were so badly washed by heavy rains shortly after planting that a very irregular stand resulted. These plots were therefore not used for the yield data. The data on stem lesions are based on one plot from each treatment in each place in 1926 and 1927 and from two plots in each place in 1928, a total of eight plots for each treatment.

The treatments, which differed somewhat each year, were as follows:

Key to 1926 Experiments

1. Standard mercuric chloride (1–1000), 2 hour soak, cold.
2. Mercuric chloride (1–500), 5 minute soak, cold.
3. Mercuric chloride (1–1000) + 1 per cent HCl, 5 minute soak, cold.
4. Mercuric chloride (1–1000) + 2 per cent HCl, 5 minute soak, cold.
5. Mercuric chloride (1–500) + 1 per cent HCl, 5 minute soak, cold.
6. Mercuric chloride (1–500) + 2 per cent HCl, 5 minute soak, cold.
7. Check. No treatment.
8. Semesan, 0.25 per cent, 5 minute soak, cold.
9. Semesan, 0.25 per cent + 2 per cent HCl, 5 minute soak, cold.
10. Hot formaldehyde (1–120), 3 minute soak, 49° C.

Key to 1927 Experiments

1. Standard mercuric chloride (1–1000), 2 hour soak, cold.
2. Mercuric chloride (1–1000), 5 minute soak, cold.
3. Hot mercuric chloride (1–1000), 2 minute soak, 49° C.
4. Mercuric chloride (1–1000) + 1 per cent HCl, 5 minute soak, cold.
5. Mercuric chloride (1–500) + 1 per cent HCl, 5 minute soak, cold.
6. Check A. No treatment.
7. Mercuric chloride (1–500) + 1 per cent HCl, 10 minute soak, cold.
8. Mercuric chloride (1–500) + 1 per cent HCl, 20 minute soak, cold.
9. Mercuric chloride (1–500) + 1 per cent HCl, 40 minute soak, cold.
10. Hot formaldehyde (1–120), 3 minute soak, 49° C.
11. Formaldehyde (1–120), 5 minute soak, cold.
12. Formaldehyde (1–120) + 1 per cent HCl, 5 minute soak, cold.
13. Mercuric chloride (1–1000) + 0.2 per cent citric acid, 5 minute soak, cold.
14. Mercuric chloride (1–1000), sat. solution of picric acid, 5 minute soak, cold.
15. Saturated solution of picric acid in water, 5 minute soak, cold.
16. Check B. No treatment.
17. Semesan Bel, No. 2, 1 lb. to 10 pints water, instantaneous dip, cold.
18. Semesan Bel, No. 21, 1 lb. to 15 pints water, instantaneous dip, cold.
19. Bayer Dipdust, 1 lb. to 20 pints water, instantaneous dip, cold.
20. Bayer Special dust, No. 181, 1 lb. to 5 gallons water, instantaneous dip, cold.

Key to 1928 Experiments

1. Standard mercuric chloride (1–1000), 2 hour soak, cold.
2. Hot mercuric chloride (1–1000), 2 minute soak, 49° C.
3. Mercuric chloride (1–1000) + 1 per cent HCl, 5 minute soak, cold.
4. Check A. No treatment.
5. Mercuric chloride (1–1000) + 1 per cent HCl, 20 minute soak, cold.
6. Mercuric chloride (1–1000) + 0.2 per cent citric acid, 5 minute soak, cold.
7. Mercuric chloride (1–500) + 1 per cent HCl, 5 minute soak, cold.
8. Check B. No treatment.
9. Hot formaldehyde (1–120), 3 minute soak, 49° C.
10. Formaldehyde (1–120) + 1 per cent HCl, 5 minute soak, cold.
11. New Improved Semesan Bel, 1 lb. to 20 pints water, instantaneous dip, cold.
12. Bayer Dipdust, 1 lb. to 20 pints water, instantaneous dip, cold.

Local tap water, which was relatively hard, was used in all experiments. The temperature of the cold solutions was approximately 10° C. Where hydrochloric acid was added to the solution, the percentage is figured in terms of volume of commercial hydrochloric acid of approximately 31 per cent strength. Thus "1 per cent HCl" means one c.c. of commercial hydrochloric acid to each 100 c.c. of the disinfecting solution.

The notes on stem lesions were taken when the plants were about 12 inches high. The plants were pulled out of the ground and washed, and the stems were examined for lesions. In the figures given, no distinction is made between light and heavy infections. If proper values had been given to different degrees of infection, the differences would have been more striking.

Notes on tuber infection and yield were taken at harvest. The tubers were thoroughly washed and those having even a few small sclerotia were considered infected. Here, also, the differences would have been more striking if consideration had been given to the different degrees of infection and to the grading qualities of the different lots.

DISCUSSION OF RESULTS

The averages of each treatment for each year are presented in graphs (Figs. 1–5) by means of which the relative value of the treatments may be observed.

From a study of the graphs it is obvious that, of those treatments which have been given sufficient trial, the standard mercuric-chloride method, the acidulated-mercuric-chloride method, and the hot-formaldehyde method gave better results than the other treatments. It is also quite obvious that none of the organic mercury compounds reduced the amount of the disease sufficiently to justify their consideration for practical use. In fact, in many

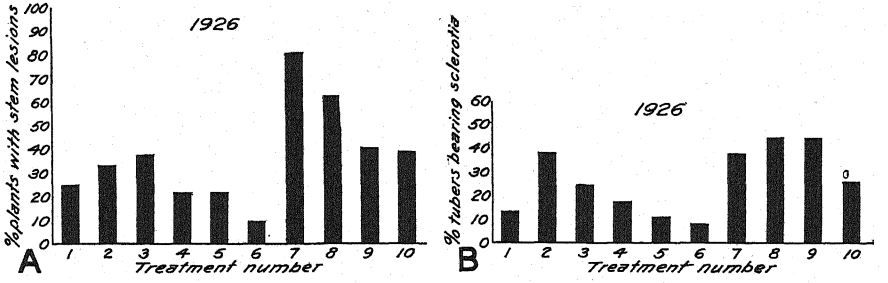


FIG. 1. A. Results of seed-tuber disinfection in 1926 in terms of average percentage of plants with stem lesions, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1926 in terms of average percentage by weight of tubers bearing sclerotia, St. Paul and Crookston.

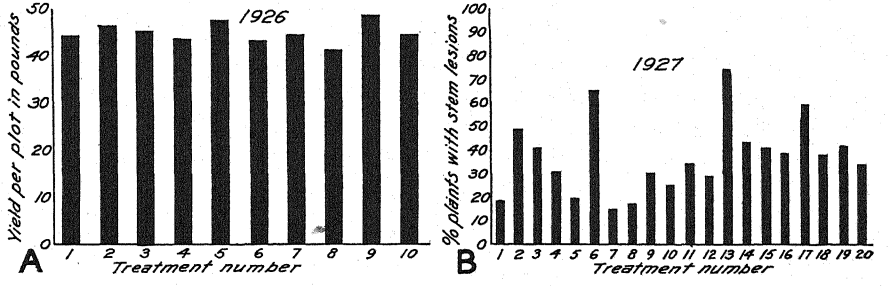


FIG. 2. A. Results of seed-tuber disinfection in 1926 in terms of average yield in pounds per plot, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1927 in terms of average percentage of plants with stem lesions, St. Paul and Crookston.

cases the yield from treated tubers was almost as badly diseased as that from the checks.

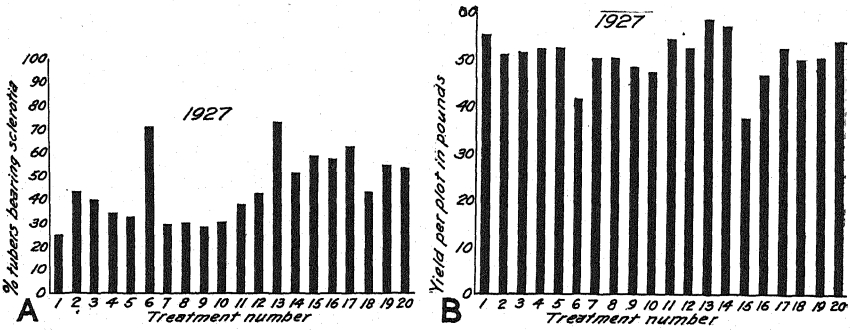


FIG. 3. A. Results of seed-tuber disinfection in 1927 in terms of average percentage by weight of tubers bearing sclerotia, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1927 in terms of average yield in pounds per plot, Crookston.

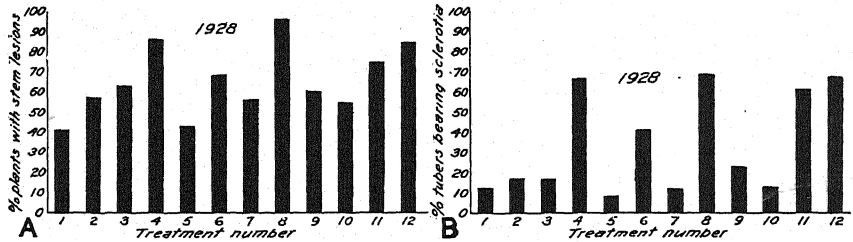


FIG. 4. A. Results of seed-tuber disinfection in 1928 in terms of average percentage of plants with stem lesions, St. Paul and Crookston. B. Results of seed-tuber disinfection in 1928 in terms of average percentage by weight of tubers bearing sclerotia, St. Paul and Crookston.

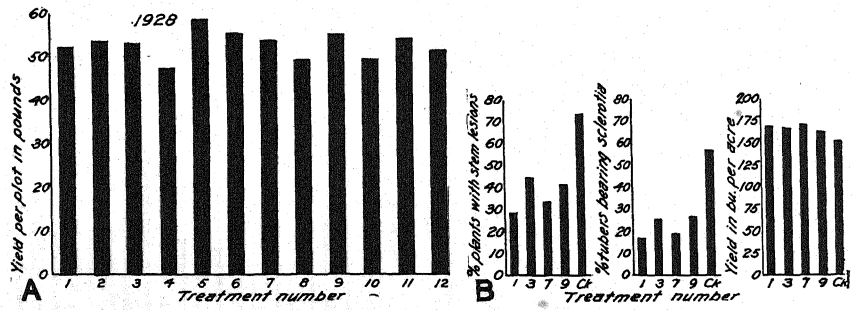


FIG. 5. A. Results of seed-tuber disinfection in 1928 in terms of average yield in pounds per plot, St. Paul and Crookston. B. Results of seed-tuber disinfection. Three-year average for the standard mercuric chloride treatment (1), acidulated mercuric chloride (1–1,000) + 1% HCl, (3), acidulated mercuric chloride (1–500) + 1% HCl, (7), the hot formaldehyde treatment (9), and the check (ck).

TABLE 1.—*The results of seed-tuber disinfection by the acidulated mercuric-chloride method (1–500) + 1 per cent HCl (7), compared with the results obtained with the standard mercuric-chloride method (1), the hot formaldehyde method (9), and the check (ck), in terms of percentage of tubers bearing sclerotia of Rhizoctonia solani*

Year and place	Treatment[a]	Percentage Rhizoctonia	Comparison with check		Comparison with (7)	
			Difference	Diff./P.E.	Difference	Diff./P.E.
St. Paul, 1926	1	9.00 ± 0.64	−26.03 ± 3.46	7.52	− 5.00 ± 2.47	2.02
	7	14.00 ± 2.39	−21.03 ± 4.12	5.07		
	9	28.23 ± 0.41	− 6.80 ± 3.46	2.00	+14.23 ± 2.42	5.88
	ck.	35.03 ± 3.43				
Crookston, 1926	1	19.03 ± 3.48	−24.34 ± 4.36	5.57	+ 9.03 ± 3.78	2.38
	7	10.00 ± 1.50	−33.37 ± 3.04	10.97		
	9	23.90 ± 1.60	−19.47 ± 3.09	6.30	+13.90 ± 2.19	6.34
	ck.	43.37 ± 2.65				
St. Paul, 1927	1	5.80 ± 0.51	−43.56 ± 1.21	36.00	− 7.56 ± 2.16	3.48
	7	13.36 ± 1.45	−36.00 ± 1.81	19.89		
	9	14.30 ± 0.69	−35.06 ± 1.30	26.96	+ 0.94 ± 1.60	0.58
	ck.	49.36 ± 1.10				
Crookston, 1927	1	43.77 ± 3.82	−34.85 ± 4.36	7.99	− 8.68 ± 5.61	1.54
	7	52.45 ± 4.12	−26.17 ± 4.58	5.71		
	9	47.60 ± 6.90	−31.02 ± 7.19	4.31	− 4.85 ± 8.03	0.60
	ck.	78.62 ± 2.03				
St. Paul, 1928	1	15.72 ± 1.67	−81.26 ± 1.75	46.43	− 1.73 ± 2.13	0.81
	7	17.45 ± 1.33	−79.53 ± 1.43	55.61		
	9	31.67 ± 2.36	−65.31 ± 2.41	27.09	+14.22 ± 2.88	4.94
	ck.	96.98 ± 0.54				
Crookston, 1928	1	10.26 ± 1.30	−26.39 ± 1.62	16.28	+ 3.66 ± 1.36	2.69
	7	6.58 ± 0.42	−30.07 ± 1.05	28.63		
	9	14.08 ± 0.93	−22.57 ± 1.34	16.83	+ 7.50 ± 1.01	7.42
	ck.	36.65 ± 0.97				
Three year average, St. Paul and Crookston. Total of 26 plots	1	16.26 ± 0.48	−39.17 ± 2.10	18.65	− 1.60 ± 0.69	2.32
	7	17.86 ± 0.50	−37.57 ± 2.11	17.80		
	9	24.75 ± 1.66	−30.68 ± 2.63	11.66	+ 6.89 ± 1.73	3.98
	ck.	55.43 ± 2.05				

[a] The numbers used for these treatments were not uniform for the three years. The numbers used in the tables are those used in 1928.

TABLE 2.—*The results of seed-tuber disinfection by the acidulated mercuric-chloride method (1–500) + 1 per cent HCl (7), compared with results obtained with the standard mercuric-chloride method (1), the hot-formaldehyde method (9), and the check (ck), in terms of total yield in pounds per plot*

Year and place	Treatment[a]	Yield	Comparison with check		Comparison with (7)	
			Difference	Diff./P.E.	Difference	Diff./P.E.
St. Paul, 1926	1	19.33 ± 0.19	+ 4.58 ± 0.34	13.47	+ 0.41 ± 0.22	1.86
	7	18.92 ± 0.12	+ 4.17 ± 0.30	13.90		
	9	17.25 ± 0.39	+ 2.50 ± 0.48	5.20	− 1.67 ± 0.40	4.17
	ck.	14.75 ± 0.28				
Crookston, 1926	1	47.13 ± 1.08	− 5.37 ± 1.92	2.80	− 5.20 ± 1.57	3.31
	7	52.33 ± 0.90	− 0.17 ± 1.68	0.10		
	9	50.00 ± 0.18	− 2.50 ± 1.53	1.63	− 2.33 ± 0.92	2.53
	ck.	52.50 ± 1.43				
Crookston, 1927	1	55.37 ± 1.03	+ 11.00 ± 1.56	7.05	+ 2.66 ± 1.07	2.48
	7	52.69 ± 0.33	+ 8.32 ± 1.21	6.88		
	9	49.69 ± 2.22	+ 3.32 ± 2.51	1.32	− 5.00 ± 2.24	1.56
	ck.	44.37 ± 1.17				
St. Paul, 1928	1	48.75 ± 0.86	+ 2.50 ± 1.02	2.45	− 2.94 ± 0.92	3.19
	7	51.69 ± 0.32	+ 5.44 ± 0.63	8.63		
	9	52.31 ± 1.58	+ 6.06 ± 1.67	3.62	+ 0.62 ± 1.61	0.38
	ck.	46.25 ± 0.55				
Crookston, 1928	1	55.43 ± 0.92	+ 5.18 ± 1.07	4.84	− 0.07 ± 1.10	0.06
	7	55.50 ± 0.61	+ 5.25 ± 0.81	6.48		
	9	57.71 ± 0.67	+ 7.46 ± 0.86	8.51	+ 2.21 ± 0.90	2.45
	ck.	50.25 ± 0.54				
Three year average, St. Paul and Crookston. Total of 21 plots*	1	50.57 ± 0.29	+ 4.80 ± 0.41	11.72	− 0.68 ± 0.37	1.84
	7	51.25 ± 0.24	+ 5.48 ± 0.39	11.50		
	9	50.35 ± 0.35	+ 4.58 ± 0.45	10.18	− 0.90 ± 0.42	2.14
	ck.	45.77 ± 0.30				

* Due to heavy, washing rains shortly after planting, the stands at St. Paul in 1927 were so irregular that the yield data were not reliable. The yield data for St. Paul in 1927 are therefore not included here.

[a] The numbers used for these treatments were not uniform for the three years. The numbers used in the tables are those used in 1928.

TABLE 3.—*The results of seed-tuber disinfection by the acidulated mercuric-chloride method (1–500) + 1 per cent HCl (7), compared with results obtained with the standard mercuric-chloride method (1), the hot-formaldehyde method (9), and the check (ck), in terms of percentage of plants with stem lesions*

Date and place	Treatment[a]	Percentage of plants with stem lesions	Comparison with check		Comparison with (7)	
			Difference	Diff./P.E.	Difference	Diff./P.E.
Average of three years at St. Paul and Crookston. Total of 8 plots	1	32.00 ± 1.60	−47.07 ± 2.25	20.92	− 6.77 ± 2.62	2.58
	7	38.77 ± 2.08	−40.30 ± 2.61	15.44		
	9	46.26 ± 2.40	−32.81 ± 2.87	11.43	+ 7.49 ± 3.17	2.36
	ck.	79.07 ± 1.58				

[a] The numbers used for these treatments were not uniform for the three years.
The numbers in the tables are those used in 1928.

In 1926, several strengths of acidulated mercuric chloride were used. Of these, the one containing 2 per cent hydrochloric acid gave the best control, but it was not enough better than the one containing 1 per cent hydrochloric acid to justify the additional cost of the material. Therefore, all subsequent experiments were made on the basis of 1 per cent-hydrochloric-acid content with different amounts of mercuric chloride and different lengths of soaking. Considering the results of the three-year period, the solution containing 1 per cent hydrochloric acid and mercuric chloride at the rate of 1–500 appears to be the most effective.

In order to evaluate more accurately the relative effectiveness of this method, the results obtained with it have been compared biometrically with those of the standard mercuric-chloride method, the hot-formaldehyde method, and the check. These comparisons are given in tables 1 to 3.

From the data presented in the graphs and the table, it may be concluded that:

1. The mercuric-chloride treatments gave better results than the hot-formaldehyde treatment.
2. The standard mercuric-chloride method resulted in slightly better control of Rhizoctonia than the acidulated mercuric-chloride method, although the difference is not strikingly significant.
3. The acidulated mercuric-chloride method resulted in yields equal to those of the standard mercuric-chloride method and slightly better than those of the hot-formaldehyde method.
4. All three of these treatments resulted in marked disease control and significant increases in yield as compared to the checks.
5. Considering the many advantages of the acidulated-mercuric-chloride method over the standard mercuric-chloride method or the hot-formaldehyde method, its performance is sufficient to justify more extensive trial under varied field conditions.

Advantages of the acidulated-mercuric-chloride method. As pointed out above, one of the greatest obstacles in the way of general use of seed-tuber disinfection is the time and trouble involved. In this respect the acidulated-mercuric-chloride method has many advantages over any method previously devised. These may be mentioned briefly as follows:

1. A soak of 5 minutes is sufficient to produce results practically equal to those of a two-hour soak with the standard mercuric-chloride method.
2. The use of hard water does not alter the effectiveness of the solution since the use of acid overcomes this.

3. Mercuric chloride dissolves very rapidly in the acidulated solution, while it dissolves very slowly and with difficulty in cold water.
4. No heating equipment is required.
5. No thermometer for temperature control is necessary.
6. There is little danger of seed injury by prolonging the time of soaking. No injury was produced by soaking as long as 40 minutes.[3]
7. With this latitude in time of soaking there is less danger of failure to control by too short exposure.
8. There is no danger of poor control by allowing the temperature of the solution to drop a few degrees.

The loss of strength of the acidulated solution. The rate of loss of acid and mercuric chloride from the solution determines the quantity of tubers which can be treated before the solution must be discarded or more acid and mercuric chloride added. In order to determine this rate of loss a solution was prepared in which seven successive lots of tubers were treated. The tubers were treated in an earthenware crock, in which just enough solution was placed to completely cover the tubers. The tubers were moderately dirty and were considered as an average seed lot. A sample of the solution was taken after each five-minute treatment. These samples were analyzed for acid and mercuric chloride content by the Division of Agricultural Biochemistry. The results are given in table 4. It is obvious

TABLE 4.—*The rate of loss of mercuric chloride and hydrochloric acid from the treating solution during the process of treating seven successive lots of tubers*

	Content of $HgCl_2$ in per cent	Content of HCl in per cent
Original solution	0.2266	0.376
After 1 treatment	0.2152	0.372
After 2 treatments	0.1997	0.353
After 3 treatments	0.2088	0.351
After 4 treatments	0.2047	0.347
After 5 treatments	0.2010	0.342
After 6 treatments	0.1974	0.338
After 7 treatments	0.1864	0.328

[3] Some seed injury has been observed in experiments made in 1929 where large quantities of tubers were treated and stored in large bins for several days before planting. Injury has also been observed where the treated tubers were planted in very dry soil. Injury in the latter case was not peculiar to the acidulated mercuric-chloride method, since every treatment used, including various modifications of the mercuric chloride and formaldehyde methods, resulted in some seed injury and delayed germination. Efforts are being made to find a means of avoiding this injury before the method is recommended for general use.

from these figures that at least eight and probably more lots of tubers could be treated in such a solution before it would be necessary to add more acid or mercuric chloride. More experiments are planned to check these results under field conditions. Such trials will be necessary also for devising the most convenient and practical procedure in applying the method.

SUMMARY

1. A solution of mercuric chloride (1–500) acidulated by the addition of 1 per cent by volume of commercial hydrochloric acid has been tested as a seed-tuber disinfectant in comparison with the standard mercuric-chloride method, the hot-formaldehyde method, and several other methods.

2. The experiments were performed at two different places in Minnesota and include data for three seasons. The data show that by using the acidulated solution as a five-minute cold soak better results are obtained than those obtained by the hot-formaldehyde method and virtually as good as those secured by the standard mercuric-chloride two-hour soak.

3. Experiments show that after treating seven successive lots of moderately dirty tubers in the acidulated solution only 17.7 per cent of the mercuric chloride and 12.2 per cent of the acid were lost.

4. The simplicity and ease of application of the acidulated mercuric-chloride method give the method many advantages over methods now in use and justify its recommendation for extensive trial under field conditions. Further experiments with the view of perfecting the method are under way.

5. Several of the organic-mercury treatments have been tested and all were found to be ineffective in reducing the amount of infection by *Rhizoctonia solani*.

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A BUTT-ROT OF BALSAM FIR CAUSED BY POLYPORUS BALSAMEUS PK.

ERNEST E. HUBERT

THE DISEASE

An interesting brown butt-rot of the balsam fir, *Abies balsamea* (L.) Mill., has been reported by Faull (1, 2) as causing serious damage in the pulpwood stands of Canada. It has also been found by the writer in the balsam-fir stands (4) of northern Wisconsin and Minnesota where its rapid development following the death of the host due to the spruce bud worm, *Cacoecia fumiferana* Clem., has largely been responsible for the fact that salvaging of such a stand after the third year is very nearly hopeless. The fungus causing this disease was noted by Peck (5, p. 46) as early as 1878 in the balsam stands of the Adirondack Mountains. Peck (6, p. 91) also collected a very similar fungus on *Tsuga canadensis* (L.) Carr., in New York, and named it *Polyporus crispellus* Pk.

The brown cubical rot, found in the butt and roots of balsam fir, is caused by *Polyporus balsameus* Pk. Three other fungi, *P. floriformis* Quel., *P. tephroleucus* Fr., and perhaps *P. crispellus* Pk., may be regarded as synonyms of the above fungus.

In 1922 the writer collected *P. balsameus* on living but suppressed trees of *Thuja occidentalis* L. in the Door-County region of Wisconsin. The butt-rot in these trees was similar to that observed in the balsam firs and no other fungus sporophore was found associated with these infections. Faull reported, in a letter to the writer, his having collected this fungus on the above host under conditions which he believed to indicate parasitism. In the Door-County cedars no evidence was obtained to indicate that the fungus was attacking the live sapwood but it was observed that the brown rot filled the entire heartwood area and left but a narrow margin of sapwood apparently unaffected. Like certain other wound fungi, under favorable conditions, *P. balsameus* may invade the sapwood zone.

SYMPTOMS

The external symptoms indicating the presence of this destructive butt-rot within the living tree are not very numerous. Whenever present, the typical sporophore of the fungus attached to roots or butt sections, is good evidence of butt-rot. At times, however, the fruiting bodies are not attached to the trunk or to exposed roots but develop on the ground at a point away from the tree where a root comes very near the surface. Under such conditions the sporophore more nearly fits the description given for

Polyporus floriformis and is characterized by a rosette-like development. This characteristic presence of fruiting bodies is in evidence only for a short period during the growing season, for insects destroy the sporophores soon after they become mature.

Basal fire scars, frost cracks, wind cracks, pin knots on lower trunk, and injuries to exposed roots are common exterior signs which indicate ease of infection by this fungus. Basal fire scars and frost cracks were nearly always noted in connection with trees showing balsam butt-rot.

Resin flow in the root crotches and along the exposed portions of roots is quite common and also serves as an excellent indicator of this rot, along with a general swollen appearance at the base of some of the trees. The

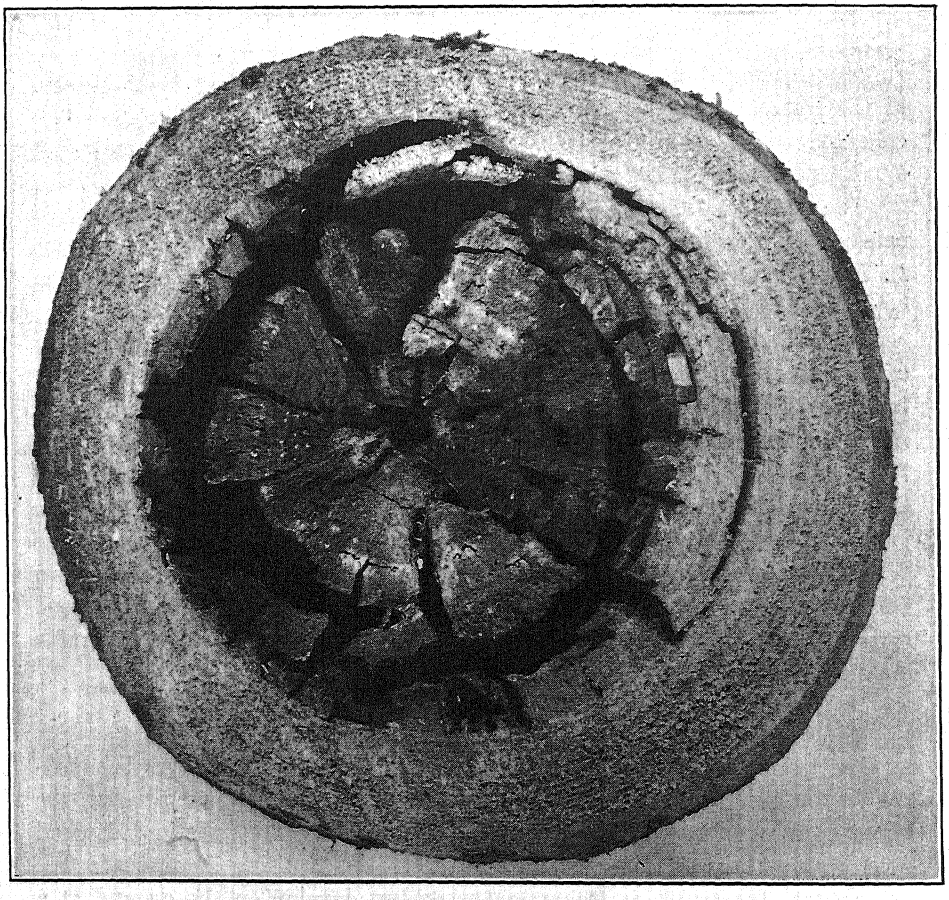


FIG. 1. The late stage of decay in *Abies balsamea* caused by *Polyporus balsameus*. This section was taken from a 55-year-old wind-thrown tree showing severely rotted roots. Note the shrinkage cracks.

typical rot in the late stage of its development, showing a downy layer of mycelium on the surfaces facing the shrinkage cracks, serves as an additional symptom, as does also the hollow sound at the butt section when the trunk is struck with an ax.

Swollen and punk knots have not been found associated with this disease.

DESCRIPTION OF THE ROT

Butt-rot of the balsam is classified under the brown-rots and is a characteristic brown, cubical rot (Fig. 1). The description of its early and late stages and the pathological histology of the infected wood is given below. The characteristics of the rot in *Thuja occidentalis* are very similar to those of *Abies balsamea.*

Early Stage. In longitudinal section, the early rot area is a warm buff, buff-yellow to light buff, fading into the whitish color of the normal wood. The wood in this stage is already somewhat softer than the noninfected wood. In this discolored area of early decay numerous hyphae are found extending into the apparently sound and normal-color wood beyond (4). In this stage the infected tissues show considerable evidence of fungous action. Some change in the cellular structure is evidenced by the use of the Bismark-brown-methyl-violet stain. Where this stain enters the cell walls they appear to be a deeper color than in the sound wood beyond. These infected tissues show numerous bore holes (Fig. 2) in the cell walls

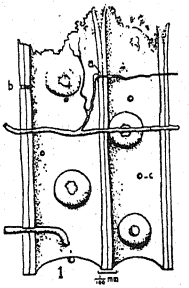


FIG. 2. Hyphae of *P. balsameus* in the wood cells of *Abies balsamea.* At a, a young thread has penetrated the wall. Bore holes produced by the fungus are shown at b and c. The decomposition of the cell wall and the corrosion and cracking of the bordered pits are prominent.

and a distinct corrosion of the surfaces of the cell wall. The cell walls are also reduced in thickness in comparison with the thickness of normal cell walls.

Late Stage. In end fracture, the color of this stage of decay is a sayal brown and when the rot is crushed to a powder it becomes a clay color. The older rot at the base of infected trees sometimes has a hazel-brown or snuff-

brown color. The cubes formed are large, the majority of the larger cracks running parallel to radial lines. The shrinkage cracks are of three types: (1) Those following annual rings, (2) those following radial lines, and (3) those in a plane traversing the stem. Within these shrinkage cracks is often formed a downy to powdery layer of white mycelium. The rotted wood crushes easily to a fine powder which feels soft and smooth between the fingers. Neither stage of decay shows zone lines.

Microscopic examination of the tissues in this stage of decay shows a very marked decomposition of the cell walls. They appear thinner, more corrosion is noted, and the bordered pits in the tracheid cells show irregular, enlarged and cracked pit openings (Fig. 2). Bore holes are numerous (Fig. 2, b, c) and the cell walls tear easily under sectioning and show ragged edges. The individual walls of the cells separate from each other in the heavily infected tissues, indicating a weakening or dissolution of the middle lamella. The reaction given when the ordinary reagents used to detect lingocellulose are applied indicates that the compound usually giving a cellulose reaction no longer responds. Apparently the cell substance remaining is composed principally of a lignin compound or has been so changed that the reaction is no longer characteristic. The hyphae are somewhat constricted where they pass through the cell walls. (Fig. 2, a.)

THE CAUSAL FUNGUS

When Koch's rules are applied to prove that *Polyporus balsameus* causes the characteristic butt-rot with which it is constantly associated, it is found that all these rules are satisfied. The fungus has been constantly found in direct association with the brown, cubical rot in the roots and basal trunks of conifers. A fungus has been repeatedly isolated from the infected wood, grown in pure culture, and inoculated under laboratory conditions on sterilized pieces of Sitka spruce wood. The fungus has produced in these pieces the rot symptoms characteristic of those of *P. balsameus*. It was later reisolated from the infected sticks and compared with the original pure cultures and with cultures obtained from the sporophores. The isolation cultures also were compared with the cultural characters for *P. balsameus* given by Miss Fritz (3).

TAXONOMY OF THE FUNGUS

Polyporus balsameus (Fig. 3) has not been collected extensively. J. R. Weir, who identified some of the early collections made in Wisconsin, probably has the largest collection of specimens from various regions yet assembled by any forest mycologist. The writer's collections are limited to sporophores and infected wood gathered at various points in northern Wisconsin and Minnesota. The sporophores appear in late August and early

September and soon disappear. This short period of fructification accounts, no doubt, for the seeming rarity of this plant. However, the sporophores are very abundant during the period of their production and vanish rapidly soon after maturity because of the attack of numerous grubs and beetles,

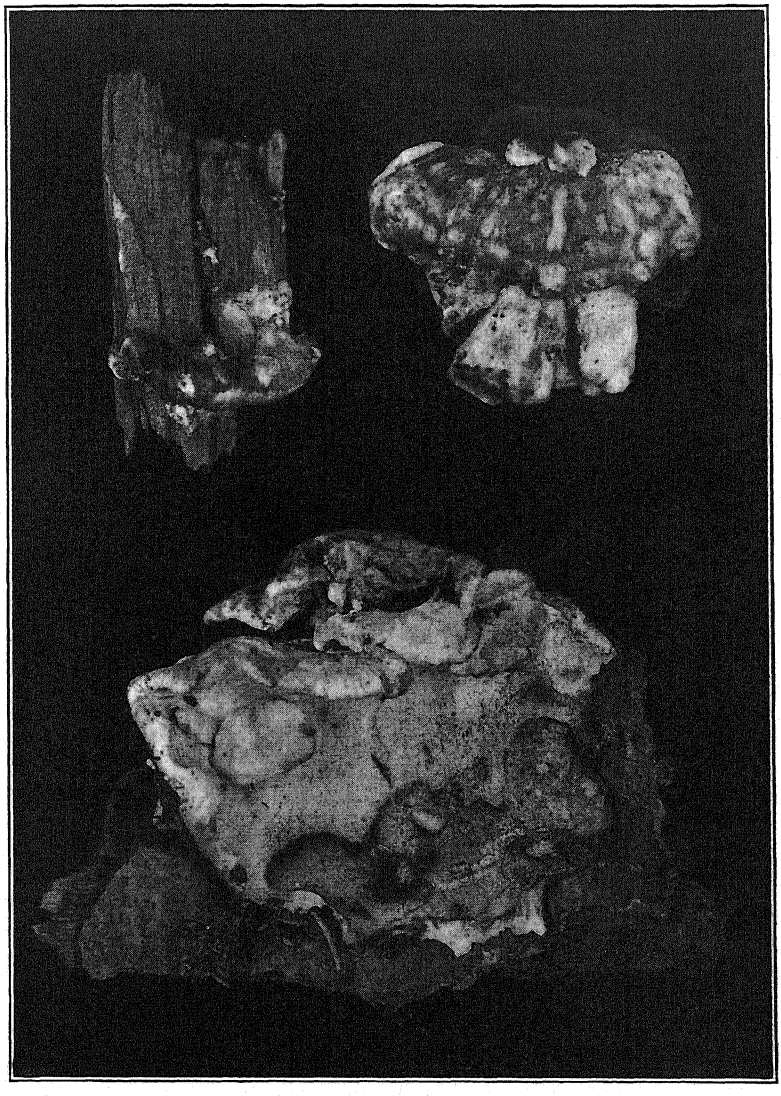


FIG. 3. Dried sporophores, upper and lower surface views, of *Polyporus balsameus* on *Abies balsamea*. The zonation of the upper surface is typical.

which completely and promptly riddle the fruiting body. There is frequently but a trace of the sporophore left attached to the tree.

The fungus develops annually. It bears a single layer of tubes distinct from the context. With the exception of the presence of cystidia and of certain variations in form and color, due mainly to local differences in habitat, Peck's description (5) may be used for this fungus:

Polyporus balsameus: "Pileus rather thin, corky, plain, about one inch broad, sessile or spuriously stipitate, slightly and unequally villose-tomentose, pale-brown, marked with lighter concentric zones; flesh white; pores short, minute, subrotund, the thin dissepiments acute, denticulate, white. Trunks of balsam trees, *Abies balsamea.* Adirondack Mts. August." (See Fig. 3, for the characteristic zonation.)

Isolation cultures from the early and late stages of rot in balsam fir were made on plain malt-agar slants. The 10-day-old cultures show white to grayish white aerial growth, fluffy at the lower end of the slant. Later this color changes to alutaceous or buff. Cultures 43 days old show a white to tan color in the lower slant while the upper slant shows a dark, reddish brown growth often somewhat horseshoe-shaped. The central area frequently shows small button-like groups of whitish hyphae which in older cultures are tipped a tan to brown color. The medium is usually stained a very dark brown color.

DAMAGE

Most of the balsam firs ranging in diameter from 3 to 18 inches, in the peninsula or Door-County region of Wisconsin were found to be infected with *Polyporus balsameus.* In the badly rotted trees the rot column was found to run from 6 to 12 feet up into the trunk in smaller trees and in lighter infections the rot column averaged 3 to 4 feet up the trunk. The rot column is usually circular in cross section of the trunk and tapers at the upper limit to a conical shape.

In the wet, "overflow" areas where rain and snow water or overflows from streams remain in hollows for considerable periods of time, many of the infected balsams were found broken by wind action either at or near the ground line or at points on the trunk ranging from 1 to 8 feet from the ground. Several trees were overturned or uprooted by the failure of the severely rotted supporting roots. These trees ranged from 4 to 20 inches in diameter.

Sporophores were most common on trees growing in very moist ground and were here found attached to exposed roots, in root crotches where frost cracks developed, and on scars or injuries to the root or root collar. They were rarely found on trees growing on slopes or well-drained ground.

In the Grand-Marais region of Minnesota conditions were observed that were very similar to those found in Wisconsin. In this region large areas of balsam and spruce trees were dead or dying from the attack of the bud worm. Many of the balsams showed balsam butt-rot and in the dead trees the rot developed rapidly, invading the sapwood and weakening the tree. As a result of this condition windfall and windbreakage were common, many of the trees being broken off at a point 2 to 6 feet from the ground. About 15 per cent of the balsam trees in this region showed butt-rot and these infected trees, in 3 to 5 years after death produced by bud-worm attack, were considered worthless by cruisers.

Windbreakage and windfall usually take place at from 1 to 3 years following the death of the trees.

The damage to potential pulpwood forests, where balsam fir is found in any appreciable amount, is reflected in the fact that second-growth stands of balsam, 50 to 60 years old, have the heartwood of the butt section and roots completely rotted. A high percentage of such infected trees are either uprooted or broken off by the wind.

SUMMARY

A brown butt-rot of *Abies balsamea* caused by *Polyporus balsameus* has been reported from Canada and the United States within recent years. The disease is a serious factor in the handling of pulp stands containing large percentages of balsam fir and particularly so in stands attacked by the bud worm, *Cacoecia fumiferana*. Symptoms of this heartwood disease are presented, including detailed, gross, and histological descriptions of the stages of decay.

Koch's rules of pathogenicity have been fulfilled in determining the fungus responsible for this butt-rot and a brief taxonomic description is given of *P. balsameus*.

It is pointed out that windbreakage and windfall are common types of loss resulting from the weakening of roots and butt sections of infected trees. This loss usually occurs at from one to three years following death of the trees due to the bud-worm attack.

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THE BLACK MOLD OF ONIONS, CAUSED BY *ASPERGILLUS NIGER* v. TIEGH.[1]

J. E. MACHACEK

During the autumn of 1928, the writer's attention was drawn to an unusual prevalence of black mold on imported Spanish onions. The epidemic appeared to be very severe and reduced considerably the market value of the vegetable. An investigation was made to determine the extent of the disease and to discover if there was any association of the causal organism with other, possibly pathogenic, bacteria and fungi.

Four shipments amounting to 40,000 cases of Spanish onions, imported from Valencia, Spain, were available for examination. These were examined when in the hold of the freighter, or while they were being unloaded into the dock warehouses, and also in cold storage. A casual examination showed the disease prevalent in each case, the percentage of diseased bulbs ranging from 10 to 100 per cent.

ISOLATIONS

An examination of the surface growth of the pathogene revealed the presence of *Aspergillus niger* v. Tiegh., but a more detailed examination of the shipments showed that some of the bulbs, though attacked by the above-named pathogen, also showed a rot, obviously of bacterial origin. Therefore, it was thought very probable that an association, such as had been described by the author in a previous paper (6), existed between the two or more pathogenes. Isolations substantiated the early microscopic examinations. Typical colonies of *Aspergillus niger*, *Fusarium* sp., *Erwinia carotovora* (Jones) Committee S. A. B., and a saprophytic Saccharomyces were obtained finally in pure culture. Of the above group only the Aspergillus and the bacterium proved pathogenic, the other two being saprophytes.

SYMPTOMS

From the examinations of many diseased bulbs it was concluded that in the majority of cases the initial invasion by the fungus began at the neck of the onion. In a few instances the diseased area originated at the base of the bulb, but the invasion of the neck, followed by a downward growth of the fungus, appeared to be the rule.

From the data available it was found that invasion of the bulbs begins at the time when some of the leaves of the plant met their death through wilting and breaking over, or after the death of the entire top at maturity,

[1] Due credit is here given to Prof. G. W. Scarth for valuable assistance given during the survey of the disease.

and then penetrates slowly into the interior of the bulb or spreads in the form of black streaks beneath the outer dry scales. In many cases it was found that, while there appeared to be no external evidence of the disease, yet, when the bulbs were cut in two, the central portion was blackened by the fungus, and usually a secondary rot had set in. Most of the bulbs examined were of peculiar construction, for it was found that an open space existed inside the bulb and extended from the base of the neck to varying depths within the bulbs. Usually this space was lined with the dried tops of the scales, and was, therefore, a suitable habitat for a weak parasite such as *Aspergillus niger*. This fungus initiated a minor rot in the bulbs, often a basis for a more destructive and extensive rot caused by *Erwinia carotovora*, which had probably invaded the plant in the wake of the above-mentioned fungus. (Fig. 1.)

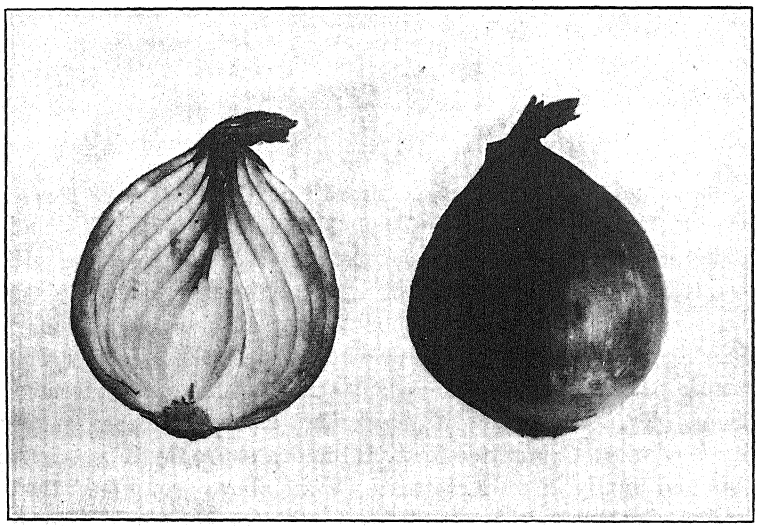


FIG. 1. Two halves of the same bulb showing open space in bulb. The fungus is present in dead scale tops.

Surface infections on the bulbs are characterized by the formation of shallow lesions on the outer scales, by the streaks of black fungous growth, and by discolored, irregular areas where the fungus had grown in bruises on the scales. (Figs. 2 and 3.) The surface infections, where there were no bruises, usually appeared as black streaks radiating from the base or the top of the onion bulbs, the most active growth of the fungus taking place along the veins of the bulb scales. (Fig. 4.)

Wayne (9) finds, in Ohio, that the disease does not confine its activities to mature bulbs, but also damages the seed and onion sets. He believes

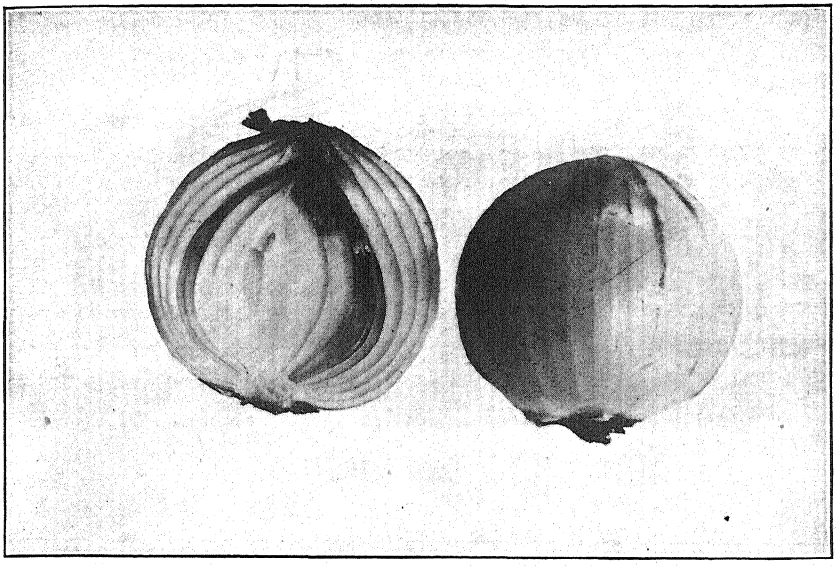


FIG. 2. Two halves of the same bulb, showing external streaking, as well as the bacterial soft rot induced by a condition shown in Fig. 1.

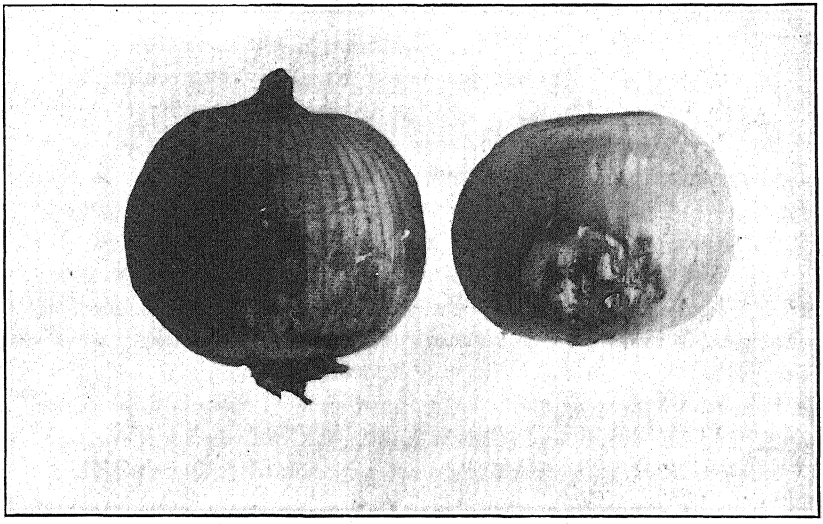


FIG. 3. Two types of surface rots caused by bruises followed by infection by *Aspergillus niger*.

(10) that the disease is carried by the seed and recommends remedies accordingly. Taubenhaus (8) finds that mature bulbs usually are most susceptible and that the infection is brought about through harvesting the

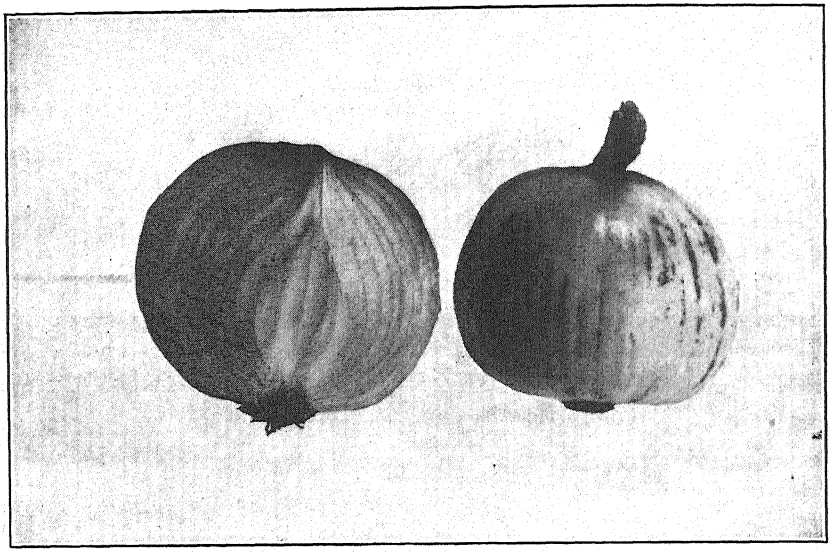


FIG. 4. Two halves of the same bulb showing outside streaking and healthy interior.

bulbs under unfavorable climatic conditions. He (8) gives the following characterization of the disease:

"The black mold first appears as blackened areas on the upper two or three layers of the bulb scales. When carefully examining these dark areas, one will find that they consist of deadened tissue, the surface of which has been blackened with loose spores of *Aspergillus niger*. As the diseased area increases, larger numbers of the bulb scales become involved and in time the entire onion shrivels and blackens and is then worthless."

The writer never has found the extreme condition mentioned above, for, though the market value of the onions was considerably reduced, unless the secondary, bacterial soft rot had made considerable progress, the bulbs were still marketable and could be kept so for a long period under proper storage conditions.

It has been observed that the amount of soft-rotted bulbs is in direct proportion to the number of bruised bulbs, this ratio being increased a good deal through the tendency of the bacterial rot to follow initial invasions of the bulbs by *Aspergillus niger*.

FACTORS INFLUENCING INFECTION

1. *Variety*.—Spanish onions appear to be attacked exclusively. This is probably due to the peculiar method of bulb maturing, with the top and center of the bulb remaining open to infection.

2. *Packing.*—Small cases and those which are loosely packed contain fewer diseased bulbs than large or tightly packed cases. In the former categories the amount of the disease was 10–50 per cent, while in the latter the amount was 40–100 per cent.

3. *Handling.*—Care in the handling of the bulbs during loading, transit, and unloading is essential. An examination showed that bruised bulbs are most liable to infection by Aspergillus, and also, in such bruises, the bacterial soft rot is almost certain to appear.

4. *Harvesting.*—Taubenhaus (8) finds that harvesting under proper conditions reduces the amount of infection considerably. He finds that during wet weather the onion bulbs become gorged with water and, in that condition, they are very susceptible to the disease. It is essential that harvesting should be done during dry weather whenever possible.

OTHER HOSTS OF THE PATHOGEN

Beille (1) observed that *Aspergillus niger* was able to induce a "stinking rot" of coffee grains.

Brooks, Fisher, and Cooley (2), working with fungi causing apple rots in storage and transit, found that *A. niger* was one of them.

Butjagin (3) reports that meat is susceptible to decomposition by *A. niger*.

Galli-Valerio and Rochaz-de Jongh (4) find that *A. niger* is pathogenic on mosquito larvae, but it is not practicable to use this fungus for the treatment of marshes and stagnant water where mosquitoes are breeding.

Hodgson (5) reports that *Sterigmatocystis* (*Aspergillus*) *niger* is able to cause an internal black "smut" of the white Adriatic fig.

Shapovalov (7) finds that *A. niger* can cause a tuber rot in Irish potatoes.

Taubenhaus (8) has been able to secure infection by the above organism on the pea, bean, tomato, carrot, pomegranate, peach, corn, and onion, and on bee larvae. In all cases the rot was severe.

There can be no doubt, therefore, concerning the ability of *A. niger* to cause disease in many other plants. As the fungus is cosmopolitan in its presence and can adapt itself to a wide range of conditions, it is probable that its weakly parasitic habits are the only explanation for its limitations as a phytopathogenic organism.

CONTROL

Wayne (10) suggests that, as a control measure, the onion seed should be sown by a drill to which an appliance is attached, capable of dripping a formaldehyde solution (one pint to 25–33 gals. water) into the seed bed along with the seed. As for the onion sets, he recommends soaking them for

six hours in a formaldehyde solution of the above strength or for the same length of time in a solution of powdered calcium hypochlorite (one-half pound in one gallon of water).

After infection takes place, the best means of preventing the disease from spreading through the bulb is to harvest the bulbs under dry conditions, dry them thoroughly after lifting, and then keep them in a cool, dry store room. Cold storage is recommended when the bulbs are to be kept for a prolonged period, as the fungus grows slowly under such conditions and the bulbs will remain in a reasonably good market condition for several months.

Care in packing and handling appears to be essential in order to retain the maximum market value of the bulbs. As bruises on the bulbs are almost certain to become infected, the prevention of these bruises by proper packing and handling is necessary.

SUMMARY

1. A bulb-rotting disease of Spanish onions induced by *Aspergillus niger* is discussed.

2. The fungus causes considerable discoloration of the bulbs, both on the outside and in the interior; and, while incapable of rotting the bulbs directly, causes a considerable decrease in the market value of the bulbs. It also enables the soft-rotting microorganism, *Erwinia carotovora*, to gain entry and begin a destructive rot of the bulb.

3. The interior of the bulb may be infected and rotted without any external evidence of the presence of the fungus.

4. Seed and set disinfection prevents most of the early field infection, but care in harvesting, handling during transit, and storing is necessary to the maintenance of the market value of the bulbs.

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STUDIES ON THE MYCELIUM OF *CRONARTIUM COMPTONIAE* ARTHUR ON *PINUS SYLVESTRIS* L.

W. G. HUTCHINSON

This study of the mycelium of *Cronartium comptoniae* Arthur has been undertaken in order to facilitate the determination of the presence of the rust upon Scotch pine in the absence of aecial fructification and other definite symptoms.

The characteristic symptoms manifested by trees infected with this rust are the swelling and cracking of the bark, the muscling of the wood, and the formation of fusiform galls (3) (4). In the early stages of the growth of the fungus, when these symptoms are not pronounced and there is no aecial fructification, it is difficult macroscopically to recognize the disease.

This study has been made on infected specimens of *Pinus sylvestris* L. collected by W. H. Snell in the neighborhood of Keesville, N. Y., in the summer of 1927.

METHODS

Portions of the bark and wood were taken from over fifty different trees. This material was then cut into small pieces not over one-half inch square and placed in a formaldehyde-alcohol mixture made by adding 6 c.c. of commercial formaldehyde to 100 c.c. of 70 per cent alcohol (1). They were kept in this fixing solution about three months and then transferred to 30 per cent alcohol. They were then put through 20 per cent, 10 per cent, 5 per cent, and 2½ per cent alcohol and finally transferred to distilled water. Before sectioning, the pieces were placed in 5 per cent gum-arabic solution for about ten minutes.

In sectioning, staining, and clearing, the author has followed the methods outlined by Colley (2) in his study of *Cronartium ribicola*. The sectioning was done with an ether freezing microtome. The sections were cut from 5 μ to 20 μ in thickness. The thicker sections proved better for tracing the mycelium between the cells, but the haustoria could be much more easily seen in the thinner sections. Most of the sectioning was done in a radial plane, since the mycelium could be more readily traced through such sections.

The method of staining with safranin and *licht Grün*, as described by Colley, was employed. This method gave excellent results. Some of the sections were stained with the Pianeze 3B stain described by Vaughan (5). With this stain, also, the mycelium and haustoria could be distinctly seen.

MYCELIUM OF THE PARASITE AND ITS PARASITISM

The mycelium of *C. comptoniae* has been found very irregular in outline, varying in width from 3.6 μ to 6.2 μ. It frequently appears swollen, forming characteristic bulges, is septate, and distinctly vacuolate. Each cell of the mycelium is uninucleate.

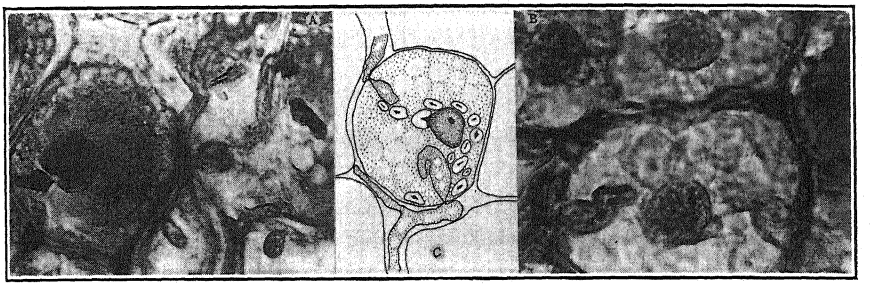


FIG. 1, A. Cells from bark of *Pinus sylvestris* infected with haustoria of *Cronartium comptoniae*. Note nucleus dented by a haustorium. ×420. B. Haustorium of *Cronartium comptoniae* showing typical bulge and bending toward the nucleus of the host cell. ×500. C. Camera-lucida drawing of cells from bark of *Pinus sylvestris* penetrated with haustoria of *Cronartium comptoniae*. Note the haustorium which has grown toward the nucleus and then has bent backward and grown toward the cell wall. Note also the numerous starch grains in the cell. ×320.

Numerous haustoria enter the cells of the bark. At the point where a haustorium passes through the wall of the host cell, it is noticeably constricted (Fig. 1, C). At this point of constriction, the haustorium measured 1 μ to 2 μ in width. The portion of the haustorium within the cell averages 5.2 μ in width and, in some parts where bulges occur, it may be as much as 8 μ in width. After penetrating the cell for some distance, the haustorium characteristically bends toward the nucleus (Fig. 1, B). Several cases were observed where the nucleus had been dented by the haustorium (Fig. 1, A). No cells have been noted in which the haustoria were branched. In a few cells the haustoria were observed growing into the cell toward the nucleus and the bending backward and toward the cell wall (Fig. 1, C). What causes this reaction it is difficult to determine. It is perhaps caused by some repelling influence in the nucleus or by some chemical substance in the cell which is antagonistic to the haustorium.

The mycelium of this fungus may be distinguished in general from that of other fungi occurring on *Pinus sylvestris* by its large size, the presence of large haustoria, and the characteristic bulges which occur chiefly on the haustoria. Its growth is intercellular. The cells adjacent to the mycelium are often pushed widely apart by the hyphae (Fig. 1, C). This probably accounts for the swelling of the bark.

Haustoria have been observed in the parenchyma cells of the cortex and phloem, in the sieve tubes, and in the ray parenchyma. The parenchyma cells of the phloem and the ray parenchyma are the principal cells attacked. In very few cases has the mycelium been found in the xylem. When it has been found there, no definite haustoria were evident; the mycelium appeared to be inside of the tracheid cells and to be growing through the bordered pits.

The cells entered by the haustoria do not appear to be greatly damaged by the fungus. In most cases they are somewhat more vacuolate than the normal cells. Most of them contain starch grains or oil globules (Fig. 1, C).

IDENTIFICATION BY MYCELIUM

Pinus sylvestris is subject to several rusts besides *Cronartium comptoniae,* namely, *Cronartium pyriforme* (Peck) Hedgc. & Long, *Cronartium cerebrum* Peck, and the Woodgate rust (3). The fact that *C. cerebrum* and the Woodgate rust have globose galls removes any possibility of confusion between *C. comptoniae* and these rusts. From microscopic study of mycelium, however, it is yet impossible to distinguish between any of these fungi. Until a comparative study of the mycelium of *C. comptoniae* and that of the other rusts has been made, the positive identification of this fungus by its mycelium is impossible. In certain cases, however, the method of identification outlined above is of value. The mycelium around the aecia has been found to correspond with that in suspected cases.

SUMMARY

1. The method of double staining of the mycelium of *Cronartium comptoniae* with safranin and *licht Grün* has been followed and found to give excellent results.

2. The mycelium of *Cronartium comptoniae* is characterized by its large size and by the possession of bulged haustoria.

3. The mycelium occurs in the parenchyma cells of cortex, phloem, and medullary rays, in the sieve tubes, and occasionally in the tracheid cells of the xylem.

4. The fungus parasitizes the cells of *Pinus sylvestris* by growing between and separating them and also by penetrating the cells with its large haustoria.

5. A comparative study of the mycelia of all the rusts occurring on *P. sylvestris* is necessary before a definite determination of the fungus by its mycelium is possible, although this method of identification is of value in certain cases.

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A ROOT AND BUTT ROT OF CONIFERS CAUSED BY *POLYPORUS CIRCINATUS* FR.

ERNEST E. HUBERT

Attention already has been directed to the work done in the forest and by means of cultures in the laboratory, in associating *Polyporus circinatus* Fr. with a white-pocket rot of roots and butt sections of conifers (2) and the comparison of pure cultures of this fungus with cultures of *Trametes pini* (Brot.) Fr. (2). During the past field season, the fungus was observed attacking the roots and butt sections of living western white pines, *Pinus monticola* Dougl., in northern Idaho. Acting as a wound fungus, it enters through injuries to the roots or root collar and produces a serious heart rot of the butt of many trees. It appears to be more prevalent in the white-pine areas where basal fire scars are common and invariably one may expect in such stands a considerable percentage of butt rot, partly caused by the above fungus and partly produced by *Polyporus schweinitzii* Fr. The fungus has also been collected in association with the typical rot in Engelmann spruce, *Picea engelmanni* Engelm., Douglas fir, *Pseudotsuga taxifolia* (Poir.) Britt., western hemlock, *Tsuga heterophylla* (Raf.) Sarg., and western larch, *Larix occidentalis* Nutt. The fungus associated with the typical rot has also been collected on *Picea mariana* (Mill.) B., S., and P. in northern Minnesota.

Previous to these observations and laboratory tests, this rot was attributed to *Trametes pini* and was assumed to be a butt-rot development of the ring-scale fungus differing from the normal development of this rot, which is commonly found in the main trunk and top of the tree.

Faull, in 1922 (1), reported a root and butt rot in spruce, hemlock, and eastern white pine caused by *Polyporus tomentosus* Fr., a synonym for *P. circinatus*. The description and habit of the sporophore correspond to those of *P. circinatus* and the rot is described as a white-pocket rot. He states that in the Otter district of Ontario a high percentage of the white pine is affected and results in considerable culling of the butt logs.

In 1927, Solovieff (5) reported a rot in spruce which he attributes to *Polystictus triqueter* Fr. This fungus is considered by mycologists to be very near if not identical with *P. circinatus* but Solovieff retains the former name because of differences in the macroscopic structure of the fruiting bodies of the two and differences in the rot descriptions. In western Russia this fungus causes considerable damage to spruce trees, the rot extending a distance of about 10 feet into the trunk from the base of the tree and for some distance into the roots. Because of the weakening of the roots and of

the butt section due to the rot, the infected trees are said to be very subject to windfall.

This is the second fungus the rot of which has been differentiated (3) from that caused by *Trametes pini*. The first fungus of this type, *Polyporus anceps* Pk., produces a white-pocket rot known as red-ray rot in the heartwood of living western yellow pine, *Pinus ponderosa* Laws., and in the heartwood and sapwood of the dead tree and its parts (4). For some time past, scalers of the Inland Empire region have called this typical top rot, "red-ring rot," and believed it to be caused by *T. pini*. Red-ray rot is a common rot of the slash and débris of western yellow pine found on logging areas in the above region.

DESCRIPTION OF DISEASE

The early-stage discoloration of red root rot caused by *P. circinatus* appears as a dark, reddish brown band surrounding the white-pocket or late decay areas. The darker color is one of the main characters distinguishing the early stage of this rot from that caused by *T. pini*. The discoloration accompanying early decay caused by *T. pini* is pinkish, reddish, or purplish, but never as dark in color as in red root rot. The wood, though darkly colored, is firm and shows no signs of pocket formation at this stage of development. Cultural studies indicate the presence of fungus threads in the discolored, early rot areas.

Numerous long, narrow and elliptical pockets filled with white fibers characterize the late stage of decay. The pockets have pointed ends similar to those found in ring rot, and these frequently run together in the direction of the grain of the wood. The wood between pockets is of a dark brownish color similar to the early rot discoloration. Unlike red ring rot, no zone lines appear in the rot and no black flecks have been noted in the pockets similar to those found in spongy sap rot, caused by *Fomes annosus* Fr., and in spongy root rot caused by *Poria subacida* Pers. (4).

Histological studies of red root rot give further evidence of the differences between this rot and that caused by *T. pini*. The hyphae of *P. circinatus* in *Picea mariana* are not constricted where they pass through the cell walls and the bore holes in the walls are considerably larger in diameter than the hyphae. Most of the hyphae run lengthwise of the wood cells; very few are observed penetrating many walls in a direct line. The hyphae of *T. pini* usually are constricted where they pass through small bore holes in the cell walls, show buckles, and penetrate several walls in a direct line.

The cultural characters of *P. circinatus* on malt-agar media differ sufficiently from those of *T. pini* on the same media to make possible their successful use in the identification of the two rots. The cultures of the former are of a much darker brown and do not show the cottony aerial growth so

common to cultures of *T. pini*. *P. circinatus* produces a subsurface growth in cultures and the surface growth is somewhat adpressed. The agar immediately beneath the mycelial mat is dark brown when viewed from the side of the tube opposite the slant or from the under side of the petri dish. The aerial hyphae of *T. pini* are light orange yellow to cinnamon buff.

SPOROPHORES

The sporophores of *Polyporus circinatus* are commonly found developing on the forest duff or on the ground some little distance away from the trunk of the infected tree. In this respect, it resembles in habit the sporophore of *P. schweinitzii* which is most commonly found on the ground. *P. schweinitzii* is sometimes found on infected logs or stumps and attached to the butt section of trees. In this environment it is truly applanate and shelf-shaped, departing from the usual circular, depressed form and central stipe. *P. circinatus* has not yet been collected on the trunk of the host but has been found attached to the buried roots by means of the mycelial strands which penetrate the duff and soil. The sporophores vary considerably in size and color with changes in environment, being larger, more regular in form, and brighter in color on moist sites and smaller, somewhat irregular in form, and darker on drier and more exposed areas. It is not found in very dry situations, since it is favored by a fairly moist environment. The sporophores may easily be mistaken by woodsmen for small or imperfectly developed sporophores of *P. schweinitzii*.

Little is known regarding the method of infection. The field observations made in Minnesota, Idaho, Washington, and Montana, indicate the fungus to be a wound organism, entering the roots through injuries of some kind and developing in the heartwood as a heart-rot agency. Correlation between fire scars at the base of the tree and on exposed parts of the roots with the entrance of this fungus has not been established, although the relationship between red root rot and basal fire injuries seems to be fairly constant.

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INACTIVATION OF THE TOBACCO-MOSAIC VIRUS BY HIGH PRESSURES

N. J. GIDDINGS, H. A. ALLARD, AND B. H. HITE

Some years ago (1915–1920), Giddings and Hite were conducting experiments to determine the effects of high pressures on various enzymes, and, at the suggestion of the senior author, in October and November, 1919, a little research was conducted on the tobacco-mosaic virus. Because of the present great interest in virus diseases and the fact that these experiments indicate possibilities of another method of learning something about them, it has seemed desirable to report on this research. The equipment and methods used have already been described in detail.[1]

Freshly expressed juice from mosaic-diseased tobacco plants was sent from Washington, D. C., to the West Virginia Agricultural Experiment Station, where the pressure equipment was located. This juice was immediately placed in an ice box where all of the samples were kept, except when they were in the press, until they were returned to Washington. All of the juice, both for use in the press and that held as control samples, was kept in block-tin tubes from the time it was received at the West Virginia Agricultural Experiment Station until it was used for inoculations in Washington.

In view of the fact that some of our other work was conducted with the materials at temperatures of 40 to 60° C., it may be well to state that all work with the tobacco-mosaic virus was at room temperatures (20–22° C.).

In all inoculation tests, young, rapidly growing Maryland Mammoth tobacco plants were used, as previous experience had shown that young plants at this stage could be used to best advantage. Ten plants were employed in each test. The results are given in table 1.

It will be noted that the twenty control plants all remained healthy; the thirty plants inoculated with the untreated mosaic juice showed twenty-six mosaic plants; the forty plants inoculated with mosaic juice which had been subjected to 75,000 lbs. pressure showed thirty-one mosaic plants; and that mosaic symptoms did not develop in any of the plants inoculated with mosaic juice which had been subjected to pressures of 130,000 lbs. or more.

The pressure required to inactivate or destroy this virus is higher than the pressure death point of *Bacillus prodigiosus* (Ehrenberg) Flugge; *Bacterium lactis aerogenes* Huppe; *Streptococcus lacticus* Kruse; *Bacillus typhosus* Eberth; *Bacillus diphtheriae* Loffler; *Saccharomyces cerevisiae*

[1] Hite, B. H., N. J. Giddings, and C. E. Weakley, Jr. The Effect of Pressure on Certain Microorganisms Encountered in the Preservation of Fruits and Vegetables. W. Va. Agr. Exp. Sta. Bul. 146. 1914.

TABLE 1.—*Summarized results which show the inactivating influence of high pressure for different periods of time on mosaic-tobacco extract*

Source of extract	Separate trials	Treatment		Total plants inoculated	Total plants infected
		Pressure	Time		
	No.	Pounds	Days	No.	No.
Healthy tobacco	2	0	0	20	0
Mosaic tobacco	3	0	0	30	26
do	2	75,000	7	20	15
do	2	75,000	5	20	16
do	2	130,000	1	20	0
do	3	135,000	2	30	0
do	1[a]	200,000	½	10	0
do	5	200,000	2	50	0
do	2[b]	200,000	7	20	0

[a] This sample was given additional treatment of ½-day at 100,000 pounds.

[b] These samples were given additional treatment of 15 hours at 100,000 pounds.

Meyer; and *Saccharomyces albicans* Reess, but lower than that of the spore-forming organism, *Bacillus subtilis* F. Cohn. It may also be noted that the enzyme zymase is inactivated or destroyed by lower pressure than that required to destroy the tobacco-mosaic virus, while pepsin is inactivated or destroyed at pressures which would seem likely to approximate those required for the tobacco-mosaic virus if its "pressure death point" had been more closely determined.

It is clearly evident that the higher pressures were effective in destroying or inactivating the virus of the tobacco mosaic and it is reasonably certain that the time-pressure line between 75,000 lbs. and 130,000 lbs. would yield further data of considerable interest. It is also highly probable that similar studies with other viruses would assist materially in their classification, by bringing out their reaction to this test.

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PHYTOPATHOLOGICAL NOTE

The relation of moisture to stomatal infection.—At the meeting of the American Phytopathological Society, in December, 1928, a paper was presented entitled "Studies on the influence of environment on infection by certain bacterial plant parasites." An abstract of the paper was published in Phytopathology.[1] This paper presented as a new discovery the fact that many more stomatal infections are obtained when plants are held in the damp chamber for 24 hours before spraying with inoculum than when they are inoculated at once on being placed in the damp chamber.

This fact has been known and utilized for many years in the Laboratory of Plant Pathology in the United States Department of Agriculture, in Washington; in fact, this has been the routine method of procedure in producing stomatal and waterpore infection.

As long ago as 1905 Dr. E. F. Smith[2] published directions for spray inoculations as follows:

". . . Stomatal infections may be secured by subjecting the plants to conditions similar to those occurring in nature on dewy nights or during heavy fogs or prolonged rains, i.e., by placing the potted plants on wet sand, atomizing thoroughly with sterile water and covering with tall, roomy bell-jars. The experiment should be undertaken in a cool rather than a warm house. When the right conditions have been obtained, moisture covers the surface of the plant in tiny drops which do not evaporate. The bell-jar may now be raised and the plant again atomized lightly with sterilized water containing the bacterium. The best time to do this is late in the afternoon, so as to take advantage of the cooler night temperature. When the bell-jar is returned, which should be immediately after spraying, it should be covered with cloth or paper to protect from the light. Usually bell-jars should be removed at the end of twenty-four hours, but exceptionally they may be left on thirty-six to forty-eight hours, if not exposed to the sun. . . ."

It will be seen that although Dr. Smith does not specify the number of hours for this preliminary treatment, he describes the condition of the plant ready for inoculation for which approximately that length of time in a damp chamber is required.—MARY K. BRYAN.—*Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.*

[1] Riker, A. J. Studies on the influence of environment on infection by certain bacterial plant parasites (Abst.). Phytopath. **19**: 96. 1929.

[2] Smith, E. F. Bacteria in relation to plant diseases. **1**: 108. Washington, D. C. 1905.

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STUDIES ON THE VARIABILITY AND DISSOCIATIONS IN THE GENUS FUSARIUM[1, 2]

LEON H. LEONIAN

PREFACE

BY C. D. SHERBAKOFF

Because of my interest in Fusaria the author of this paper has asked me to write a brief statement as a preface to his work, kindly giving me full freedom to say whatever I please.

I consider the data presented in this work to be a distinct contribution to our knowledge of the behavior of fungi. The introduction and discussion present matter of great interest to me, though I disagree with many of the conclusions and surmises, often characteristic of the mental attitude of many who spend much time on the taxonomy of Fusaria but whose main interest is in other subjects.

The experiments herein reported and the results obtained were initiated and are discussed primarily from the point of view of their bearing upon the taxonomy of Fusaria; hence, this phase must be given primary consideration.

A classification of organisms, to be of fundamental value, must aim at their genetic grouping. In the case of Fusaria, at least for the time being, we have at our disposal only indirect means for the determination of their true relationship; namely their morphological and physiological characters. Therefore, the more that is known of these characters the better shall we be prepared for a correct classification. Hence, the value of the present paper. In regard to the inadequacy of the present classification here referred to, however, it should be remembered that, so far, only a few men have given systematic attention to this subject and that the first work of value on Fusaria appeared only eighteen years ago.

There is no question that the variability of Fusaria is thoroughly demonstrated by the experiments as reported in this paper and thus they emphasize further the similar findings of other workers. The variability is, how-

[1] Published by the permission of the Director of the West Virginia Agricultural Experiment Station as Scientific Paper no. 56.

[2] The major part of the cost of publishing this paper was borne by the West Virginia Agricultural Experiment Station.

ever, primarily of the "presence and absence," and of the "more and less" type, which usually is of a barking rather than a biting kind. It will often be found more apparent than real, especially in the case of spore characters. This was considered with some emphasis a while back by the author of the preface.[3] While a greater emphasis may well be placed on the conditional variability, the facts now stand in general as they were then.

The experiments herein reported also bring out clearly the fact that organisms, similar in their morphology, usually show a similarity in certain physiological characters and thus, in the identification of Fusaria, physiological characters could be, whenever necessary, used in place of, or supplementary to, morphological characters.

The exceptions to the uniform response, the so-called erratic or inconsistent behavior as observed in all of the tests employed herein, will probably be found, after a thorough revision of the facts involved, either conditional or showing true differences in the organisms concerned.

The appearance of "mutations," "saltations," and "variants," in pure cultures of Fusaria and other fungi, is at present unquestionable. Their significance may be of some genetic and therefore of fundamental taxonomic value, but their importance in the practical identification of certain individual organisms seems to be overemphasized here and in some other works on the subject. At present, when we are quite ignorant of how properly to grow fungi for identification purposes under adequately controlled conditions, the Fusaria must be grown on media known to be most suitable to them. Especially should they be grown on media not too rich in available nitrogen and sugars. These media ordinarily do not cause the fungi to saltate, but thus support unchanged and for a long time the growth of Fusaria of the same kind as the original. For that reason, the so-called natural media are, for the time being, more suitable for morphological studies of Fusaria than the synthetic media though in their composition the former are more variable than the latter.

In regard to the questions and suggestions often heard about the need of radical improvements and changes in the taxonomy of Fusaria, it may be stated that while these are much needed and may finally be quite drastic, they can come only when additional knowledge discloses the true facts and reveals the falsity of the present assumptions. However, the practical need may, and probably will, promote sufficient study of the fungi to devise more dependable and accessible means of identification of individual Fusaria.

The author's suggestion that as many strains as possible of the same species from different parts of the world should be studied is good; however, there is the prerequisite that some dependable means be employed by which the strains may first be properly grouped into the different species. At

[3] Sherbakoff, C. D. Fusaria of potatoes. Cornell Memoir 6: 111–113. 1915.

present, however poor the presentation may be, only the morphological means are known. And here I wish to state that while the details of the present taxonomy of Fusaria are unsatisfactory, its foundation is, in my opinion, sound, though certainly it might be advantageously reinforced by the study of physiological characters.

INTRODUCTION

Variability is a most outstanding character in the genus Fusarium. It has been responsible for many complications which have arisen not only in taxonomy but in pathological relationships, as well. While some excellent work done recently both in this country and abroad has considerably clarified the situation, much more remains to be done.

The species may be highly plastic; yet, notwithstanding its fluctuations and temporary or permanent deviations from the common path, it retains some characteristic landmark whereby its relationships can be traced. Very often such landmarks are in the form of an intangible something which cannot be properly established unless the approximate limits of the variability of the organism are first defined. However, this variability is not a simple phenomenon but the expression of a complex mechanism which has received hitherto but little attention from mycologists. Numerous facts indicate that a given bacterium or fungus may not possess just a single orbit within which certain standardized phenomena manifest themselves in logical sequence, but that two or more orbits of unlike nature may be found woven into the intricate pattern of the protoplasm. Furthermore, any one of these phases may emerge and be perpetuated at the expense of all others provided the proper stimulus be present. The dissociation of these various phases in a given bacterial or fungous colony may be observed in sector formations and in the conflicting responses which the organism often makes under the most strictly controlled laboratory conditions.

The present studies are an attempt to follow the variability of Fusaria from growth reactions, and to untangle, if possible, the continuity of the specific path, thus making some contribution towards the ultimate goal, namely, the crystallization of the species concept.

It was soon observed, as expected, that many of the Fusaria began to form sectors when grown in petri dishes. Transfers made from most of these sectors gave pure cultures of the "new" strain. Large numbers of such strains were isolated in pure cultures and added to the original group of Fusaria. They can be distinguished throughout this paper by the Roman numerals accompanying their names. For example, *F. semitectum XVIII* denotes the eighteenth variant of this particular species.

All organisms were carefully checked in order to reduce any chance contamination to an absolute minimum. When one is constantly working

with nearly three hundred organisms, many of which possess spores capable of being easily carried by air currents, no amount of precaution can assure total freedom from contaminations. Nor can one be certain that cultures coming from other laboratories are always true to name. Consequently, it was found necessary to enlist the aid of C. D. Sherbakoff. After all of the experimental work was concluded, the cultures were submitted to his critical study. As a result of his findings, seven of the variants were discarded because they were believed to be contaminations; nineteen other cultures were eliminated because they were misnamed, and finally twelve more were removed from the general list because they were originally received as unidentified forms and it was deemed best to retain nothing but named species.

The writer doubts if the sectors which appeared in the colonies of Fusaria are true mutations; in fact, he doubts if mutations ever do occur in fungi. He is inclined to agree with some bacteriologists that such phenomena, at least in microbiology, are dissociations rather than mutations. It is very difficult to explain reversible behaviors in the light of mutations, whereas dissociations admirably fit into such conflicting activities. The term variant has been employed here to differentiate the new phases resulting from dissociative phenomena.

Not all of the variants described in this paper are fixed. Although some have been in culture for over three years without any noticeable change in their appearance, too many others have manifested a remarkably reversible fluctuation. In case of some organisms almost every transfer yielded a complex colony with well-defined sectors none of which could be isolated no matter how often the work was repeated. For example, regardless of whether the inoculum was taken from the colorless or colored sectors, the ensuing colony was always complex. In some others, the sectors were readily isolated in pure culture, but after "coming true" for a few months or even a year, they either completely reverted to the old type or again yielded a complex colony. When within a comparatively short period a given fungus fluctuates back and forth a number of times between two morphologically and physiologically different types, notwithstanding the fact that all environmental conditions are kept uniform, the investigator finds no means, other than dissociative phenomena, with which to explain the puzzling situation. This phenomenon will be stressed later in this paper.

Whenever more than one strain of a given organism is listed, it is designated with an Arabic numeral, such as *F. trichothecioides 5*. If such an organism has yielded variants, it is qualified by both Arabic and Roman numerals: for example, *F. trichothecioides 3–II*, which denotes the variant no. II of the strain no. 3. This scheme has been found very convenient for a simple but speedy differentiation of a large number of different forms.

The writer wishes to express his thanks to all his colleagues and friends who sent him cultures, advice, and even sympathy, all of which were sorely needed. He is especially indebted to C. D. Sherbakoff who, from the very beginning, manifested a sympathetic and active interest in the progress of the work. He not only sent cultures, revised the nomenclature, and examined the fungi used in this work, but also critically read the manuscript and made many valuable suggestions.

A considerable proportion of the tedious laboratory routine was shared with the writer by Daisy Chapman and Herbert Geer, for whose efficient assistance I wish to express my deep appreciation.

Finally, I wish to acknowledge gratefully the work of W. E. Rumsey, whose excellent photography materially contributes to this paper.

ORGANISMS, THEIR SOURCES, AND THEIR VARIANTS

An attempt was made to secure not only many different species, but also as many different strains of the same organism as possible. Most of the cultures used in this work, secured through the courtesy of C. L. Shear and Margaret Wilcox, represent the Fusaria which formed the basis of the studies by the Fusarium Conference (10). Another group of organisms came from Holland, and the remainder were secured from miscellaneous sources. As a rule, very few of the tubes showed signs of mites, Actinomyces, or bacterial contaminations. Whenever such foreign organisms were observed, the cultures were freed from them before any experimental work was begun.

The following list represents the different species, varieties and strains of Fusaria that were used in these experiments:

F. acuminatum Ell. & Eve. emend Wr. no. 1. Secured from Shear.

F. acuminatum no. 2. Isolated from sugar beets by Ernest Reed; identified by Sherbakoff. This organism has yielded eight variants.

F. alluviate Wr. & Reink. Secured from Shear.

F. anthophilum (A. Br.) Wr. Secured from Shear.

F. aquaeductuum pusilum Wr. Obtained from Holland.

F. aquaeductuum volutum. Obtained from Holland.

F. arcuatum Berk & Curt. Secured from Shear. According to Sherbakoff this organism is different from *F. arcuatum* Sherb.

F. asclerotium (Sherb.) Wr. Sent by L. R. Jones, who secured the original culture from Wollenweber.

F. aurantiacum (Link.) Sacc. Secured from Shear.

F. batatatis Wr. no. 1. Isolated from sweet potato; sent by L. L. Harter.

F. batatatis no. 2. Obtained from Holland.

F. bulbigenum Cke. & Masse. Secured from Shear.

F. bullatum Sherb. Secured from Shear. This organism has yielded two variants.

F. bullatum brevis Wr. & Reink. Secured from Shear.

F. camptoceras Wr. & Reink. Secured from Shear. This organism has yielded two variants.

F. caudatum Wr. Secured from Shear.

F. chlamydosporum Wr. & Reink. Secured from Shear. This organism yielded three variants.

F. chenopodium (Tulm.) Sacc. Sent by Sherbakoff.

F. coeruleum (Lib.) no. 1, Sacc. Sent by Freeman Weiss, who isolated it from a potato plant found at State College, Pa. This organism has yielded two variants.

F. coeruleum no. 2. Sent by Weiss, who isolated it from a potato tuber from North Dakota.

F. coeruleum no. 3. Obtained from Holland.

F. commutatum Sacc. Obtained from Holland.

F. conglutinans Wr. Sent by L. R. Jones.

F. cubense E. F. Sm. no. 1.

F. cubense no. 2. This organism was sent from Holland under the name of *F. musae* Drost. Sherbakoff believes it to be *F. cubense.*

F. culmorum (W. G. Sm.) Sacc. no. 1. Obtained from E. C. Stakman. This organism has yielded two variants.

F. culmorum no. 2. Sent by W. E. Maneval.

F. culmorum nos. 3, 4, 5 and 6. Sent by Ernest Reed, who isolated them from sugar beets. Identified by Sherbakoff. Nos. 3, 4 and 6 have yielded two variants each.

F. decemcellulare Brick. Secured from Shear.

F. dimerum Penz. Secured from Shear.

F. diversisporum Sherb. Secured from Shear.

F. ensiforme Wr. & Reink. Secured from Shear.

F. eumartii Carp. no. 1. Sent by Weiss, who isolated it from potato plant at Wanamaker, Pa. This organism has yielded two variants.

F. eumartii no. 2. Sent by R. W. Goss. This organism has yielded two variants.

F. eumartii no. 3. Sent by R. P. White.

F. euoxysporum Wr. Obtained from Holland.

F. falcatum App. & Wr. Secured from Shear.

F. filiferum (Preuss) Wr. Secured from Shear. This organism has yielded two variants.

F. flocciferum Cda. Obtained from Shear.

F. fructigenum Fr. Sent by Sherbakoff.

F. fructigenum maius Wr. forma. 1, Wr. & Reink. Secured from Shear.

F. fructigenum maius no. 2. Secured from Shear under the label of *F. stilboides* Wr. According to Sherbakoff it should be *F. fructigenum maius.* This organism has yielded seven variants.

F. fructigenum maius no. 3. Isolated by Valleau and identified by Sherbakoff. This organism yielded three variants.

F. gibbosum App. & Wr. Sent by Maneval.

F. graminum Cda. no. 1. Obtained from Holland labeled *F. limosum* Rost. According to Sherbakoff it should be *F. graminum.*

F. graminum no. 2. Isolated from lettuce by Valleau and identified by Sherbakoff. This organism has yielded two variants.

F. herbarum (Cda.) Fr. no. 1. Obtained from Holland labeled *F. putrefaciens;* according to Sherbakoff it should be *F. herbarum.*

F. herbarum no. 2. Obtained from Holland labeled *F. metachroum imperfectum* Wr.

F. herbarum gibberalloides Wr. Secured from Shear. This organism has yielded two variants.

F. herbarum pirinum (Fr.) Wr. Isolated by Ernest Reed from sugar beets and identified by Sherbakoff. This organism yielded two variants.

F. hyperoxysporum Wr. no. 1. Secured from Shear.
F. hyperoxysporum no. 2. Sent by R. F. Poole.
F. incarnatum (Desm.) Sacc. Secured from Shear. This organism has yielded four variants.
F. javanicum Koord. no. 1. Secured from Shear.
F. javanicum no. 2. Obtained from Holland.
F. lini Bol. Sent by Otto Elmer.
F. lateritium Nees. Isolated by H. S. Fawcett and identified by Sherbakoff. This organism has yielded two variants.
F. lolii (W. G. Sm.) Sacc. Secured from Shear.
F. loncheceras Sid. Sent by C. P. Sideris.
F. loncheceras microsporum Sid. Sent by Sideris.
F. longipes Wr. & Reink. Secured from Shear.
F. lutulatum Sherb. Secured from Shear. This organism has yielded two variants.
F. lycopersici (Sacc.) Wr. no. 1. Secured from Shear.
F. lycopersici no. 2. Sent by E. E. Clayton.
F. malli Taub. Secured from Shear
F. macroceras Wr. & Reink. Secured from Shear.
F. martii App. & Wr. no. 1. Secured from Shear.
F. martii no. 2. Secured from Shear.
F. martii no 3. Sent by Sherbakoff labelled *F. ipomoeae* (Halst.) Wr. In a later communication Sherbakoff writes that "there is no name of this kind but *Hypomyces ipomoeae,* the conidial stage of which much resembles *F. martii.* This organism has yielded two variants.
F. martii minus Sherb. Secured from Shear.
F. martii phaseoli Burkh. no. 1. Sent by J. E. Weimer. This organism has yielded three variants.
F. martii phaseoli no. 2. Obtained from W. H. Burkholder. This organism has yielded two variants, but Sherbakoff thinks that variant no. 2–II is some other fungus. Yet, because this form has appeared repeatedly in single-spore cultures, and, furthermore, because its reactions bear a very close similarity to those of the other strains of this fungus, the organism is retained in this work.
F. martii pisi Jones. Sent by Fred Jones.
F. martii viride Sherb. Secured from Shear.
F. moniliforme Sheld. no. 1. Secured from Shear.
F. moniliforme no. 2. Secured from Shear.
F. moniliforme no. 3. This and the following two cultures were sent by Panos Caldis, who isolated them from the interior of the fig fruit in California.
F. moniliforme nos. 4 and 5.
F. moniliforme erumpens Wr. & Reink. Secured from Shear. This organism has yielded two variants.
F. moniliforme minus Wr. & Reink. Secured from Shear.
F. moniliforme subglutinans Wr. & Reink. Secured from Shear.
F. neoceras Wr. & Reink. Secured from Shear.
F. niveum E. F. Sm. no. 1. Obtained from E. C. Sherwood, who isolated it from watermelon in West Virginia.
F. niveum no. 2. The original of this culture was sent to Sherwood from Oregon.
F. orthoceras App. & Wr. Secured from Shear.
F. orthoceras triseptatum Wr. Secured from Shear.
F. ossicolum (Berk.) Sacc. no. 1. Secured from Shear.

F. ossicolum no. 2. Obtained from Holland under the label of *F. falcatum cespitosum* Wr. According to Sherbakoff it should be *F. ossicolum.*

F. oxysporum Schl. no. 1. Secured from Shear.

F. oxysporum no. 2. Secured from Shear.

F. oxysporum nicotianae John. Secured from Shear.

F. paspalicola P. Henn. Sent by Sherbakoff.

F. pirinum Fr. Secured from Shear.

F. poae (Peck) Wr. Isolated from mustard roots by B. B. Higgins and identified by Sherbakoff. This organism has yielded two variants.

F. polymorphum Matr. Secured from Shear.

F. pusillum Wr. Secured from Shear.

F. radicicola Wr. Secured from Shear.

F. rhizochromatistes Sid. Obtained from Holland.

F. rhizochromatistes microsclerotium Sid. Obtained from Holland.

F. sambucinum no. 1, Fuckl. Secured from Shear.

F. sambucinum no. 2. Obtained from Holland labeled *F. discolor triseptatum.* According to Wollenweber this fungus is same as *F. discolor* which in turn is a synonym of *F. sambucinum* Fuckl.

F. scirpi Lamb. & Fautr. Secured from Shear.

F. sclerostromaton Sid. Obtained from Holland.

F. semitectum Berk. & Rav. no. 1. Secured from Shear. This organism has yielded eighteen strains. Sherbakoff thinks that variant 1–III does not belong here, yet because in all of its reactions it closely resembles the other strains, it is not eliminated from this paper.

F. semitectum no. 2. Secured from Shear.

F. solani (Mart. p.p.) App. & Wr. no. 1. Sent by J. J. Taubenhaus, who isolated it from spinach in Texas.

F. solani no. 2. Isolated from sugar beets by Ernest Reed and identified by Sherbakoff. This organism has yielded two variants.

F. solani no. 3. Isolated from onions by George K. K. Link and identified by Sherbakoff. This organism has yielded three variants.

F. solani no. 4. Isolated from lettuce by Valleau and identified by Sherbakoff. This organism has yielded two variants.

F. solani minus Wr. no. 1. Secured from Shear. This organism has yielded two variants.

F. solani minus no. 2. Isolated from lettuce by Valleau and identified by Sherbakoff. This organism has yielded two variants.

F. solani suffuscum Sherb. Secured from Shear.

F. spinaceae Sherb. no. 1. Sent by Sherbakoff.

F. spinaceae no. 2. Sent by Taubenhaus, who isolated it from spinach.

F. striatum Sherb. no. 1. Secured from Shear. This organism has yielded two variants.

F. striatum no. 2. Secured from Shear.

F. striatum no. 3. Isolated from lettuce by Valleau; identified by Sherbakoff. This organism has yielded two variants.

F. sulphureum Schlecht. no. 1. Sent by Sherbakoff.

F. sulphureum no. 2. Obtained from Holland labeled *F. genevense* Dasc.; according to Sherbakoff it should be *F. sulphureum.*

F. sulphureum no. 3. Obtained from Holland.

F. theobromae App. & Strk. Secured from Shear.

F. tracheiphilum Wr. Obtained from Holland.

F. trichothecioides Wr. no. 1. Sent by Weiss, who isolated it from potato tubers in Outlook, Montana.

F. trichothecioides no. 2. Sent by R. W. Goss. This organism has yielded three variants. Sherbakoff thinks that variant 2–II does not belong here, but it has been retained by the writer because in its reactions it is quite similar to the other strains.

F. trichothecioides no. 3. Sent by Otto Elmer. This organism has yielded two variants.

F. trichothecioides no. 4. Sent by R. P. White. This organism has yielded two variants.

F. trichothecioides no. 5. Sent by Maneval.

F. trichothecioides no. 6. Obtained from Holland labeled *F. tubivorum;* according to Sherbakoff it should be *F. trichothecioides.*

F. trichothecioides no. 7. Sent by J. L. Weimer, who isolated it from bean roots. Identified by Sherbakoff. This organism has yielded two variants.

F. tricinctum (Cda.) Sacc. no. 1. Secured from Shear. This organism has yielded eight variants.

F. tricinctum no. 2. Obtained from Holland. This was originally described as *F. muntzii* Wr.

F. uncinatum Wr. Obtained from Holland.

F. urticearum (Cda.) Sacc. Secured from Shear. This organism has yielded two variants.

F. vasinfectum Atk. Secured from Shear.

F. viride (Lehm.) Wr. no. 1. Secured from Shear.

F. viride no. 2. Obtained from Holland. This organism was originally described as *F. solani medium* Wr.

F. viticola Thum. Secured from Shear.

F. zonatum (Sherb.) Wr. no. 1. Secured from Shear.

F. zonatum no. 2. Sent by Taubenhaus labeled as *F. cepae* Hanz. According to Sherbakoff *F. cepae* should be *F. zonatum.*

F. zonatum no. 3. Sent by L. R. Jones as the original *F. cepae* of Walker and Tims.

MEDIA AND TECHNIQUE EMPLOYED AND TESTS APPLIED

Uniform conditions were maintained throughout these experiments, and no effort was spared to make the quality and the quantity of nutrient agars and solutions as nearly the same as possible. Unless otherwise stated, all cultures were kept at a temperature of 25° C. for two weeks before readings were made. The test-tubes and petri dishes used were uniform in size. The size and age of the inoculum were kept uniform by the following method: Fresh colonies of the fungi were grown in petri dishes and small discs of agar and mycelium were then cut by a cork borer of 5 mm. opening. A row of such discs was cut on a circular line around the colony approximately 5 mm. back of the outermost edge of growing hyphal tips. This region rather than the outermost edge of the colony was used because the young hyphae of the more rapidly growing organisms produce very sparse and superficial growth, and cannot be cut and transferred without greatly disturbing the mycelium. No such practice was found necessary in case of organisms which failed to make colonies larger than 40 mm. in diameter in six days, because such slow-growing organisms have a firm grip on the agar, even at the outermost edge of the growing zone. This method provided a rapid and very simple scheme whereby large numbers of inoculum-discs

could be cut, and each disc lifted and transferred by a platinum needle to any desired container of agar or solution.

Only one nutrient agar was used for stock cultures and it consisted of the following ingredients:

Dry malt extract	10 grams
Dextrose	10 "
Potassium nitrate	2 "
Dihydrogen potassium phosphate	1 gram
Magnesium sulfate	1 "
Shredded agar	20 grams
Distilled water	1,000 c.c.

All the ingredients were cooked together in the autoclave for fifteen minutes at fifteen pounds pressure, then tubed or plated by pouring 10 c.c. in each test-tube (16 x 150 mm.) and 15 c.c. in each petri dish (100 mm. in diameter and 15 mm. deep). The nutrient agar was then sterilized at fifteen pounds pressure for fifteen minutes.

The relative value of each reaction obtained with any of the organisms has been expressed by Roman numerals. Except where the reading stands at 0 on the one hand and I–V on the other, the comparative value of the symbols should not be stressed very much. It is unwise to assume that because an organism under identical conditions shows a growth reaction which is interpreted as I, and another under the same conditions gives a growth reaction estimated as V, there can really be a fundamental difference between the two organisms, or at least one of sufficient merit to be used in differentiative work. This may, perhaps, sound a bit too drastic, but it should be realized that while very often minute details may be of great scientific value, they are perhaps as often sources of much confusion and erroneous conclusions. Consequently, nothing but the high points in the reactions of these Fusaria have been considered.

Simplicity of experiments and elimination of the personal factor were attempted in conducting these studies. After a considerable number of preliminary tests it was decided that growth reaction would give the most dependable results. An inoculum, transferred to a certain solution, either grows or does not grow. In either case the results can be readily seen and interpreted without difficulty or confusion. While it was a comparatively easy matter to prepare solutions which would induce a good growth, much greater difficulty was encountered in a search for growth-inhibiting substances. A variety of supposedly toxic compounds was tried and discarded. It seems that Fusaria have considerable ability to tolerate many such substances in comparatively high concentrations.

One naturally thinks that such a simple thing as growth reaction would show a dependable consistency; but, as revealed in the following tables, a

great number of conflicting results have been encountered in every experiment. It became necessary, therefore, to repeat each experiment at least once, and more often two or three times. Some have been repeated a dozen times. In addition to this, the work was so planned as to check one experiment against others of the same nature, thus attempting to approach a condition where the balance between the organisms and their environment was of such nature as to render fluctuating reactions much less common. Yet, the writer refrains from making any generalization except one: the only constant thing in Fusaria is their inconstancy.

The following is a list of experiments and characteristics with which an attempt has been made to test the variability of Fusaria:

1. Rate of growth of colonies. 2. Production of color and spore masses on five different nutrient agars. 3. Sporulation on malt extract agar. 4. Growth at 5° C., 34° C., and 37.5° C. 5. Growth in presence of nine different concentrations of tartaric acid. 6. Reproduction in presence of nine different concentrations of tartaric acid. 7. Growth in presence of two concentrations of tannin. 8. Growth in presence of two concentrations of mercurochrome. 9. Growth in presence of five concentrations of malachite green. 10. Growth in presence of two concentrations of iodic acid. 11. Growth in presence of one concentration of acid phenol acetic. 12. Growth in presence of two concentrations of sodium dinitrophenoxide. 13. Growth in presence of three concentrations of sodium tribromphenate.

RATE OF GROWTH OF COLONIES

Petri dishes containing 15 cc. of malt extract agar were inoculated and kept at 25° C. for six days, and the diameter of the ensuing colonies was then measured. The results are presented in table 1. The number of measurements accompanying each organism represents the number of times the work was repeated and should embrace the scope of variability characteristic of each organism.

The colors of the six-day-old colonies are given in column 3 of table 1, not with a view to segregating color fluctuations, but because colors are considered as a somewhat significant by-product of the main experiments. The more detailed accounts on pigment formation are given in table 2. No attempt has been made to give the exact shades of mycelium or substratum because at a given time such a terminology might describe the color of a given fungus, but if the work be repeated a sufficiently large number of times, a most disconcerting fluctuation would be revealed. Such different general colors as blue-green, red, purple or violet and lavender may, at one time, be exhibited by the same strain; but upon subsequent trials the fungus may appear without any pigment whatsoever.

Other growth habits, such as the presence or absence of aerial mycelium, its nature, whether fluffy, or appearing as twisted strands, yielding or

TABLE 1.—*Rate of growth and color of colonies on malt extract agar (6 days at 25° C.)*[4]

Organisms	Measurements of Rate of Growth (mm.)	Average Rate of Growth (mm.)	Color
F. acuminatum I	90–90–90–90	90	0
F. acuminatum 2–I	100–100–100–100	100	Red-purple
F. acuminatum 2–II	55–65–90–85–90	77	Pink zone or none
F. acuminatum 2–III	50–60–90–85–90	75	Pink zone
F. acuminatum 2–IV	80–80–75	78	Pink zone
F. acuminatum 2–V	65–55–65	62	0
F. acuminatum 2–VI	85–75–80–75	79	0
F. acuminatum 2–VII	80–80–90–90–85	85	0
F. acuminatum 2–VIII	70–70–90–90–90	82	0
F. alluviale	60–70–60	63	0
F. anthophilum	70–70–65–55	65	0
F. aquaeductuum pusilum	20–25–20	23	0
F. aquaeductuum volutum	20–25–20–20	22	0
F. arcuatum	45–45–45–30	41	0
F. asclerotium	85–80–90–80	84	0
F. aurantiacum	90–85–90–80	86	0
F. batatatis 1	65–70–70–70	69	0
F. batatatis 2	80–90–85	85	0
F. bulbigenum	85–80–85–80	82	0
F. bullatum I	85–85–90–75	84	0
F. bullatum II	65–65–80–90	75	0
F. bullatum brevius	100–90–90–90	92	0
F. camptoceras I	80–80–90–80	82	0
F. camptoceras II	85–85–85	85	0
F. caudatum	90–90–90–85	89	0
F. chlamydosporum I	80–85–90–90	86	Yellow-brown center
F. chlamydosporum II	65–75–85–75	75	Orange
F. chlamydosporum III	90–90–85–90	88	Yellow-brown center
F. chenopodinum	90–85–85–90	87	0
F. coeruleum 1–1	65–60–65–65	64	Greenish yellow
F. coeruleum I–II	60–65–70–65	65	Patches of blue-green or none
F. coeruleum 2	60–65–60	62	0
F. coeruleum 3	25–40–35–30–25	31	Blue-green patches
F. commutatum	80–80–85	82	0
F. conglutinans	65–65–60–70	65	0
F. cubense 1	85–75–85–80	81	0
F. cubense 2	70–70–75	72	0
F. culmorum 1–I	100–100–95	98	Purple-red or none
F. culmorum 1–II	75–75–80	77	0
F. culmorum 2	100–100–100	100	Purple-red
F. culmorum 3–I	90–100–80–80–80	86	Flesh and yellow or red-purple or none
F. culmorum 3–II	90–100–90–100	95	0
F. culmorum 4–I	100–100–100	100	Outer zone of pink or none
F. culmorum 4–II	90–100–95	95	Red-purple
F. culmorum 5	90–95–100–100–95	96	Light red-purple outer zone
F. culmorum 6–1	75–85–90–80	82	0
F. culmorum 6–II	90–90–90–100	92	Red-purple
F. decemcellulare	45–50–50–55	50	Purple-red or none

[4] 0 indicates total absence of pigment.

TABLE 1.—(*Continued*)

Organisms	Measurements of Rate of Growth (mm.)	Average Rate of Growth (mm.)	Color
F. dimerum	60–55–60–60	59	0
F. diversisporum	80–85–70	78	0
F. ensiforme	50–50–45–50	49	Blue-green
F. eumartii 1–I	40–35–30–35	35	Blue-green or blue-green patches
F. eumartii 1–II	40–30–35–30–60–60	42	0
F. eumartii 2–I	35–40–40–40	39	Blue-green
F. eumartii 2–II	35–40–45–40	40	0
F. eumartii 3	40–35–40–40	39	0
F. euoxysporum	80–75–70–80	76	0
F. falcatum	100–90–90–90	92	0
F. filiferum I	90–100–90	93	0
F. filiferum II	100–85–90	92	Flesh
F. flocciferum	65–65–65–70	66	0
F. fructigenum	40–35–40–40	39	Flesh
F. fructigenum maius I	45–45–50–45	46	0
F. fructigenum maius 2–I	40–45–40–45	42	Red-purple to none
F. fructigenum maius 2–II	50–40–40–45	44	Flesh
F. fructigenum maius 2–III	50–40–40–45	44	0
F. fructigenum maius 2–IV	45–40–45–45	44	0
F. fructigenum maius 2–V	40–35–35–45	39	Orange
F. fractigenum maius 2–VII	40–40–40	40	0
F. fractigenum maius 2–VIII	40–35–45	40	0
F. fructigenum maius 3–I	90–80–75–60–65	74	0
F. fructigenum maius 3–II	70–85–75–75–60	73	0
F. fructigenum maius 3–III	75–75–85	78	Flesh or none
F. gibbosum	100–100–100–90	97	0
F. graminum 1	100–100–90–90	95	0
F. graminum 2–I	90–100–90	93	0
F. graminum 2–II	90–100–85	92	0
F. herbarum 1	30–35–30–30	31	0
F. herbarum 2	70–65–75–60	67	0
F. herbarum gibberalloides I	55–75–55–55	60	0
F. herbarum gibberalloides II	65–45–50–45	51	Red-purple, or red purple zone or none
F. herbarum pirinum I	40–35–40–40	39	0
F. herbarum pirinum II	50–50–60–60	55	0
F. hyperoxysporum 1	90–85–85	87	0
F. hyperoxysporum 2	80–70–75–85	77	0
F. incarnatum I	90–90–90	90	0
F. incarnatum II	90–90–85	89	0
F. incarnatum IV	70–85–90	82	Flesh
F. incarnatum V	90–90–85	89	0
F. javanicum 1	55–60–60–65	60	0
F. javanicum 2	75–100–90–80–80	85	0
F. lini	65–60–60–70	64	0
F. lateritium I	40–30–40	37	0
F. lateritium II	50–60–60	57	0
F. lolii	50–45–50–45	47	0
F. loncheceras	85–85–85–85	85	0
F. loncheceras microsporum	80–85–75–75	79	0
F. longipes	80–85–90–85	85	0

TABLE 1.—(*Continued*)

Organisms	Measurements of Rate of Growth (mm.)	Average Rate of Growth (mm.)	Color
F. lutulatum I	75–75–80–70	75	0
F. lutulatum II	75–75–80–65	74	0
F. lycopersici 1	75–75–70–85	76	0
F. lycopersici 2	80–85–85–80	82	0
F. malli	100–80–75–90	86	0
F. macroceras	100–90–90	93	Red-purple
F. martii 1	60–65–60–70	64	0
F. martii 2	90–85–85–90	87	0
F. martii 3–I	55–60–60–65	60	0
F. martii 3–II	55–60–60–65	60	Blue-green or none
F. martii minus	60–65–65–70–75	67	0
F. martii phaseoli 1–I	35–35–30–30–35	33	Blue-green or none
F. martii phaseoli 1–II	35–30–30–65–65–60	47	0
F. martii phaseoli 1–III	35–30–35–35–35	34	0
F. martii phaseoli 2–I	30–35–35–35	34	Blue-green or none
F. martii phaseoli 2–II	30–40–35–35	35	0
F. martii pisi	55–80–80–80	74	0
F. martii viride	60–75–70–70	69	0
F. moniliforme 1	85–80–80–75	80	Flesh
F. moniliforme 2	70–65–70–80	71	0
F. moniliforme 3	25–30–30–35–35	31	0
F. moniliforme 4	75–75–70–75	74	0
F. moniliforme 5	75–80–70–65	72	0
F. moniliforme erumpens I	70–50–60–70–60	62	0
F. moniliforme erumpens II	80–70–75–65–70	72	Blue-green band or none
F. moniliforme minus	75–75–80–85	79	0
F. moniliforme subglutinans	65–60–65–75	66	0
F. neoceras	55–60–55–55	56	Purple or none
F. niveum 1	75–70–75	73	0
F. niveum 2	80–90–75–85	82	0
F. orthoceras	80–80–85–80	81	0
F. orthoceras triseptatum	100–80–80–85	86	0
F. ossicolum 1	75–90–85–95	86	0
F. ossicolum 2	85–100–90–100–100	95	0
F. oxysporum 1	80–90–90–90	87	0
F. oxysporum 2	100–85–80–90	89	0
F. oxysporum nicotianae	85–80–80–85	82	0
F. paspalicola	65–65–65	65	0
F. pirinum	65–85–80–70	75	0
F. poae I	50–50–60–50	52	Red-purple
F. poae II	50–80–75–85	72	0
F. polymorphum	60–65–60–55	60	Flesh
F. pusillum	70–70–65–70–65	68	Blue-green patches or none
F. radicicola	60–70–70–75	69	0
F. rhizochromatistes	80–85–85–90	85	0
F. rhizochromatistes microsclerotium	80–85–85–85	84	0
F. sambucinum 1	40–40–40–35	39	Flesh
F. sambucinum 2	100–100–100	100	0
F. scirpi	85–80–85–80	83	0

TABLE 1.—(*Continued*)

Organisms	Measurements of Rate of Growth (mm.)	Average Rate of Growth (mm.)	Color
F. sclerostromaton	80–80–80–80	80	0
F. semitectum 1–I	50–65–75–70–65	65	Orange
F. semitectum 1–II	75–80–60–65–70	70	0
F. semitectum 1–III	75–70–65–70–65	69	Flesh
F. semitectum 1–IV	70–65–75–65–65	68	Orange-pink
F. semitectum 1–V	80–70–75–90–65	76	0
F. semitectum 1–VI	80–70–75–90–55–60	71	0
F. semitectum 1–VII	80–75–70–70–65	72	Orange or none
F. semitectum 1–VIII	90–80–85–90–80	85	0
F. semitectum 1–IX	70–70–65–60–60	65	Orange
F. semitectum 1–X	100–100–90–90–90	94	0
F. semitectum 1–XI	75–70–65–60–60	66	0
F. semitectum 1–XII	90–80–65–60–60	71	Flesh
F. semitectum 1–XIII	80–70–60–55–55	64	0
F. semitectum 1–XIV	90–85–80–70–70	79	0
F. semitectum 1–XV	80–75–80–65–65	73	0
F. semitectum 1–XVI	75–70–70–60–60	67	0
F. semitectum 1–XVII	100–90–100–85–90	93	0
F. semitectum 1–XVIII	90–100–100–90–90	94	0
F. semitectum 2	75–65–70–80	72	Pink
F. solani 1	65–70–75–80	72	0
F. solani 2–I	90–90–85–90–85	88	0
F. solani 2–II	85–70–65–70	72	0
F. solani 3–I	50–60–60–60	57	Blue-green zones or patches
F. solani 3–II	60–60–60–60	60	0
F. solani 3–III	55–60–60–65	60	0
F. solani 4–I	55–60–60–60	59	0
F. solani 4–II	55–60–60–60	59	Blue-green or none
F. solani minus 1–I	55–60–70–65–70	64	Occasional lines of blue-green
F. solani minus 1–II	60–75–60–65–70	66	0
F. solani minus 2–I	60–65–60–60	61	0
F. solani minus 2–II	65–65–60–65	64	0
F. solani suffuscum	65–60–60–65	62	Pale greenish-yellow
F. spinaceae 1	75–75–80–75	76	0
F. spinaceae 2	70–75–70–70	71	0
F. striatum 1–I	60–65–65–70	65	0
F. striatum 1–II	60–60–65–70	65	Blue-green
F. striatum 2	60–65–60–65	62	Patches of blue-green
F. striatum 3–I	60–60–60–65	61	Blue-green patches
F. striatum 3–II	65–65–60	63	0
F. sulphureum 1	90–90–100–100	95	0
F. sulphureum 2	65–75–65	68	0
F. sulphureum 3	80–85–85	83	0
F. theobrome	60–70–70–65	66	0
F. tracheiphilum	75–80–80	78	0
F. trichothecioides 1	85–60–75–70–65	71	0
F. trichothecioides 2–I	80–85–80–85–75	81	0
F. trichothecioides 2–II	70–85–85–80–85	81	Orange
F. trichothecioides 2–III	80–75–80–60	74	0
F. trichothecioides 3–I	85–85–90	87	0

TABLE 1.—(*Continued*)

Organisms	Measurements of Rate of Growth (mm.)	Average Rate of Growth (mm.)	Color
F. trichothecioides 3–II	50–40–70–75–75	62	0
F. trichothecioides 4–I	70–90–70–80	77	Light brown or none
F. trichothecioides 4–II	70–90–85–90	84	0
F. trichothecioides 5	85–80–80–75	80	0
F. trichothecioides 6	80–90–85	85	0
F. trichothecioides 7–I	60–65–60–60–65	62	0
F. trichothecioides 7–II	60–50–55–65–65	59	0
F. tricinctum 1–II	75–75–70–60	70	Red-purple patches
F. tricinctum 1–III	80–75–65–60	70	Purple-red
F. tricinctum 1–IV	55–70–60–75–60	64	0
F. tricinctum 1–V	85–60–75–65	71	0
F. tricinctum 1–VI	70–60–60–60	62	0
F. tricinctum 1–VII	75–70–80–40–65	66	0
F. tricinctum 1–VIII	60–75–50–60–60–65	61	0
F. tricinctum 1–IX	50–65–30–45	66	Pink outer zone
F. tricinctum 2	30–30–30–45	34	0
F. uncinatum	65–60–70–80–70	69	0
F. urticearum I	60–60–60–55	59	Orange
F. urticearum II	50–50–50–40	47	0
F. vasinfectum	70–70–60–55–65	64	0
F. viride 1	65–70–70–70	69	Blue-green patches
F. viride 2	65–90–80–85–90	82	0
F. viticola	55–70–55–65–70	63	Flesh
F. zonatum 1	80–80–75–80	79	0
F. zonatum 2	85–85–80–85	84	0
F. zonatum 3	85–85–90–90	86	0

resistant to the needle, and other minor details were found too untrustworthy to be included in any of the tables.

Rate of growth has always been considered a dependable specific character; yet, the foregoing table reveals a number of exceptions. *Fusarium acuminatum* 2–V averages only 62 millimeters in diameter, while the average for no. 2–I is 100 mm. *F. coeruleum* 3 measures only 31 mm., while *F. coeruleum* 1–II grows more than twice as fast. While *F. fructigenum maius* 1 and all the variants of no. 2 are comparatively slow-growing organisms, all three strains of *F. fructigenum maius* 3 grow nearly twice as fast. *F. herbarum* 1 forms a colony with a diameter of only 31 mm., while no. 2 averages 67 millimeters. *F. lateritium* I is a slow grower and no. II is a comparatively fast grower. *F. moniliforme* 3 is the only slow grower of all the strains of this species. The strains of *F. semitectum* vary from 64 to 94 millimeters, thus precluding the possibility of forming any common meeting ground for the individuals of this species. All strains of *F. eumartii* are slow growers, averaging between 35 and 40 mm. However, *F. eumartii* 1–II, after behaving like the others for a while, suddenly jumped to 60 mm. *F. martii phaseoli* 1–II has shown a similar behavior and after averag-

ing no more than 30–35 millimeters in six days, it suddenly began to measure 60–65 mm. Some of the variants of *F. semitectum* 1 also have exhibited from time to time similar sudden acceleration of growth. The possibility of contamination as a factor in such behavior does not hold because such fluctuations are reversible. Furthermore, all other characteristics of such erratic organisms have remained singularly stable, in so far as the average for the species is concerned.

PIGMENT FORMATION

Five nutrient agars have been used in order to obtain a more comprehensive range of color production and pigment fluctuation among Fusarium cultures. Of these the first three are prepared from standardized products, while the last two (steamed rice and oatmeal agar) are included to satisfy those who have been in the habit of considering complex vegetable media as the more "natural" substrata. The following formulae give the preparation of these nutrient agars:

1. *Malt extract agar.*—(Formula on page 14.)
2. *Synthetic agar.*—

Potassium nitrate	2 g.
Dihydrogen potassium phosphate	1 g.
Magnesium sulfate	1 g.
Levulose	30 g.
Agar-agar	20 g.
Distilled water	1,000 c.c.

3. *Proteose-peptone agar.*—

Proteose peptone	5 g.
Dihydrogen potassium phosphate	1 g.
Magnesium sulfate	1 g.
Maltose	20 g.
Agar-agar	20 g.
Distilled water	1,000 c.c.

4. *Steamed rice.*—
   2.5 grams of polished rice and 5 c.c. of distilled water were placed in each test-tube and autoclaved at 15 pounds for 15 minutes.
5. *Oatmeal agar.*—
   Prepared the usual way, except that only 50 grams of oatmeal was used for each 1,000 c.c. of water.

Readings were made one month after inoculation and, as far as possible, the range of variation is given in table 2, although nothing but general terms have been used in color differentiation.

Inconstancy of color production is very evident in the foregoing table. For example, *F. acuminatum* 1–I and 1–VIII, *F. asclerotium*, *F. aurantiacum*, *F. batatatis* 2, *F. culmorum* 1–I and 1–II, *F. fructigenum maius* 1–I

TABLE 2.—*Color production on malt extract, potassium nitrate, peptone and oatmeal agars, and on steamed rice (one month at 25° C.)*[5]

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. acuminatum 1	0	0	0	0	0
F. acumination 2–I	0–red	Red	Red	Yellow and brown	Red
F. acuminatum 2–II	0–orange	Orange and red	Red-yellow	Yellow and brown	Red or faint purple
F. acuminatum 2–III	0–red	Orange and red	Red-orange	Yellow and light purple	Reddish purple
F. acuminatum 2–IV	Red	Red	Red	Yellow and purple brown	Red
F. acuminatum 2–V	0	0	0	Yellow	0–faint pink
F. acuminatum 2–VI	0	0–trace of red	0–trace purple	Orange or faint purple	0–faint pink
F. acuminatum 2–VII	0	0	0	Tan–trace of purple	0–faint pink
F. acuminatum 2–VIII	Orange–faint red	0–red	0–red	Red or orange	Red
F. alluviale	0	0	0	0–pink	0
F. anthophilum	0	0–yellow	Orange	Orange	Orange
F. aquaeductuum pusilum	Flesh	Flesh	Flesh	Cream	Cream
F. aquaeductuum volutum	0	0	0	Orange	Cream
F. arcuatum	0–blue green patch	Yellow–trace of blue green	Blue green	Yellow or pink and yellow	Greenish yellow
F. asclerotium	0	0–light purple	0–light purple	0–reddish purple	Purplish gray
F. aurantiacum	0	0–lavender	0–faint purple	Purple	Purple
F. batatatis 1	0	0	0	0	0
F. batatatis 2	0	0–light purple	0	Pink	Light purple
F. bulbigenum	0	0	0	Light purple	Purple patches
F. bullatum I	0	0	0	Salmon or faint purple	Brownish yellow
F. bullatum II	0	0	0	Brown	Orange
F. bullatum brevius	0	0	0	Brown–trace of salmon	Flesh
F. camptoceras I	0–brown	Light brown	Light brown	Brown	Tan or purplish brown
F. camptoceras II	Light brown	Light brown	Light brown	Brown	Tan or trace of red
F. caudatum	0	0	0	0	0
F. chlamydosporum I	Brown	Brown	Tan–reddish purple	Light brown	Brown–trace of red

[5] 0 indicates absence of pigment.

TABLE 2.— (*Continued*)

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. chlamydosporum II	Orange	Brown and salmon	Tan-orange	Brown and salmon	Purplish brown and salmon
F. chlamydosporum III	Brown	Brown-orange	0–brown and faint purple	Light brown and faint purple	Brown–trace of red
F. chenopodinum	0	0	0	0	0
F. coeruleum 1–I	Blue-green	0–blue-green patches	0–blue-green	Pink and brown	Blue-green
F. coeruleum 1–II	0–streaks of blue-green	0–blue-green patches	Pink	Pink	0–purple
F. coeruleum 2	0	0	0	Faint pink	0–faint pink and blue-green
F. coeruleum 3	Blue-green	Light purple–trace blue-green	Blue-green	Pink	Purple–blue-green
F. commutatum	0	0	0	0–orange	0–patches of pink and orange
F. conglutinans	0	0	0	Faint purple	0
F. cubense 1	0	0–faint purple	0–light purple	0–trace of purple	Light purple
F. cubense 2	0	0–faint purple	Light purple	Reddish purple	Purple
F. culmorum 1–I	0–red	Red	Red	Gold and trace of purple	Red
F. culmorum 1–II	0	0–red and orange	0–reddish purple	Yellow and orange	Orange with red patches
F. culmorum 2	Red	Red and orange	Red	Purplish red and orange	Red and yellow
F. culmorum 3–I	Reddish purple	Red and orange	Red and orange	Gold and pink	Red
F. culmorum 3–II	0–orange	0	0	0	0
F. culmorum 4–I	Brown or red	Red and orange	Red and orange	Yellow to faint pink or purple	Red and brownish yellow
F. culmorum 4–II	Reddish purple	Red	Red	Gold and pinkish brown	Red-orange and red
F. culmorum 5	0–purple	Red and orange	Red and orange	0–yellow and trace of red	Red
F. culmorum 6–I	0	0–faint purple	0	Light purple	0–faint purple
F. culmorum 6–II	Purplish red	Crimson	Red	Yellow and pink	Red-gold and red
F. decemcellulare	0–crimson	Crimson and orange	Red	Gold or red and yellow	Red and gold
F. dimerum	0	0	0	0	0
F. diversisporum	0	0	0	0	0
F. ensiforme	Blue-green	Blue-green	Blue-green	Purplish brown	Purple

TABLE 2.— (Continued)

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. eumartii 1–I	Blue-green	0–blue-green patches	Blue-green	Faint pink	0
F. eumartii 1–II	0	0	0	Pink and brown	Blue-green and pink
F. eumartii 2–I	0–blue-green	Grayish blue patch	0–blue-green	0–light purple	0
F. eumartii 2–II	0	0	0–blue-green	Pink	0
F. eumartii 3	0	0	0	Purple	Light purple
F. euoxysporum	0–light purple patch	Faint purple	0	0–flesh	0–light brown
F. falcatum	0	0	0	Brown-purple	Brownish yellow
F. filiferum I	0–light brown	**Brown**	Light brown	0–brown and pink	0–orange
F. filiferum II	0–orange	0–orange	0	Purple	0–faint purple
F. flocciferum	0	0	0	Patches of yellow	Light yellow
F. fructigenum	0	0	0	Purplish red	Purple
F. fructigenum maius I	0	0	0	0	0
F. fructigenum maius 2–I	0–red	0–red	0	0	0–trace of red
F. fructigenum maius 2–II	0–orange	0–red	0–red patch	Yellow–faint pink	Yellow–trace of red
F. fructigenum maius 2–III	0	0	0	0	0
F. fructigenum maius 2–IV	0	0	0	0	0
F. fructigenum maius 2–V	0–orange	Orange	Orange–patch of blue-green	Salmon–light purple	0–orange
F. fructigenum maius 2–VII	0	0	0	0	0
F. fructigenum maius 2–VIII	0	0	0	0	0
F. fructigenum maius 3–I	0	0	0	Orange	0
F. fructigenum maius 3–II	0	0	0	Orange	0
F. fructigenum maius 3–III	0–orange	0–orange	0	Red–brownish purple	Faint pink
F. graminum 1	0	0	0	0	0
F. graminum 2–I	0	0	0	Brown and flesh	0–patch of red
F. graminum 2–II	0	0	0	Brown and flesh	0–patch of pink

TABLE 2.— (Continued)

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. herbarum 1	Purple-red zone	Red–trace of pink	Pink–trace of red	Gold	Red and gold
F. herbarum 2	0	0	0	0–yellow and faint pink	0
F. herbarum gibberalloides I	0	0	0	0	0
F. herbarum gibberalloides II	Red	Red-gold and red	Red	Gold and brown	Gold and red
F. herbarum pirinum I	0	0	0	0	0
F. herbarum pirinum II	0	0	0	0	0
F. hyperoxysporum I	0	0–purple	0	Faint purple	Purple
F. hyperoxysporum 2	0	0	0–trace of purple	Light purple	0–orange
F. incarnatum I	0	0	0	0–flesh	0–orange
F. incarnatum II	0	0	0	0–faint purple	0
F. incarnatum IV	0	0	0	0–salmon	0–orange
F. incarnatum V	0	0	0	0–salmon	0–yellow
F. javanicum 1	0	0	0	0	0
F. javanicum 2	0	0–purple	0–purple	Reddish purple	Purple
F. lateritium I	0	0	0	0–light purple	0
F. lateritium II	0	0	0	0	0
F. lini	0	0–lavender	0	Light purple and pink	0–faint purple
F. lolii	0	0	0	0	0
F. loncheceras	0	0–mauve	0	Light purple	Purple
F. loncheceras microsporum	0	Purple	0–purple	Light purple	Purple
F. longipes	0	0	0	0	0
F. lutulatum I	Tan–blue-green streaks	Orange–blue-green	Orange–blue-green	Yellow	Tan and purple patch
F. lutulatum II	Tan–blue-green streaks	Tan–patch of blue-green	Tan–blue-green	0	0
F. lycopersici 1	0	0	0–purple	Pink	Faint purple
F. lycopersici 2	0	0	0	0	0
F. malli	0	0	Bluish gray	Pink	0–purple
F. macroceras	Red	Red and orange	Red and orange	Gold and trace of salmon	Red
F. martii 1	0–blue-green patch	Tan–blue-green patch	Faint bluish gray–brown	Pink and brown	Yellow and faint purple
F. martii 2	0	0–faint purple	Purple–faint purple	Crimson	0–red

TABLE 2.— (Continued)

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. martii 3–I	0	0	0	0–light purple	0
F. martii 3–II	0–blue-green	0	0	Light purple	0
F. martii minus	0	0	0–trace of blue-green	0–faint purple	0
F. martii phaseoli 1–I	Blue-green	Blue-green	Blue-green	Pink and light purple	Blue-green–pink
F. martii phaseoli 1–II	0	0	0	0–faint purple	0
F. martii phaseoli 1–III	0–blue-green patch	Light purple	Blue-green–pink	Pink	0-patches of blue-green and pink
F. martii phaseoli 2–I	0–blue-green	Blue-green	Blue-green	Pink and purple	Blue-green and pink
F. martii phaseoli 2–II	0	0	0	0–pink	0
F. martii pisi	0	0	0	0	0
F. martii viride	0	0–blue-green	0–blue-green	0–light purple	0–yellow patch
F. moniliforme 1	0	0–patches of blue	0–patches of blue-green or purple	Reddish purple patch	Purplish gray
F. moniliforme 2	0	0	0	0	0
F. moniliforme 3	0	0	0	Orange	0
F. moniliforme 4	0–blue-green patch	Purple–blue-green	0–blue-green patch	Purple and red	Purple
F. moniliforme 5	0	0–purple	0	Light purple	Purple
F. moniliforme erumpens I	0	Faint purple-blue-green	Faint purple-blue green	Trace of purple	Purple and red
F. moniliforme erumpens II	0	Faint purple-blue-green	Faint-purple-blue green	Pink	Purplish gray
F. moniliforme minus	0	Purple–faint blue zone	Purple-blue-green	Reddish purple	Purple
F. moniliforme subglutinans	0–blue-green patch	0–purple	Purple-patch of blue-green	Pink	Purple
F. neoceras	0–lavender	Deep purple	Purple	Deep reddish purple	Deep violet blue
F. niveum 1	0	0–faint purple	Faint purple-patch of blue-green	Red and purple	Light purple
F. niveum 2	0	0–faint purple	Red-purple	Red and purple	Purple-patches of purple
F. orthoceras	0	0–lavender	0–light purple	Reddish purple	Purple
F. orthoceras trisepta-tum	0	0–purple	0–light purple	Faint purple-red	Purple
F. ossicolum 1	0	Greenish-yellow	0	Tan and faint pink	0–brownish yellow
F. ossicolum 2	0	0	0	Tan-brown	0–tan
F. oxysporum 1	0	0–light purple	0–purple	Purplish red	Purple
F. oxysporum 2	0–lavender patch	0–purple	0–pink	Pink	Light purple

TABLE 2.— (*Continued*)

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. oxysporum nicotianae	0	0	0	Pink	0–lavender
F. paspalicola	0–faint purple	0–trace of purple	Pink	0	0
F. pirinum	0	0	0	Orange	Yellow
F. poae I	Reddish purple	Red	Red–red and gold	Gold–gold and red	0–red
F. poae II	0–reddish purple zone	0–patches of red	0–red and gold	Yellow-gold and red	Gold and red
F. polymorphum	0	0	0	0–faint pink and yellow	0–red
F. pusillum	0	0	0	Pink	0
F. radicicola	0	0	0–patch of blue-green	0–faint purple	0
F. rhizochromatistes	0	Light purple	0–light purple	Purple	Purple
F. rhizochromatistes-micorsclerotium	0	Purple	0–purple	Purple	Purple
F. sambucinum 1	Red	Red	Red	Yellow	Red and yellow
F. sambucinum 2	0	Pinkish brown	0–brown	Orange	Brown
F. scirpi	0	0	0	0	0
F. sclerostromaton	0	Purple	0–purple	Reddish purple	Purple
F. semitectum 1–I	Orange	Orange-salmon	Salmon	Brown and salmon	Salmon
F. semitectum 1–II	0–orange	Tan	Salmon	Salmon	Orange
F. semitectum 1–III	0	0	0	0	0
F. semitectum 1–IV	Salmon pink	Salmon	Salmon	Brown and salmon	Salmon
F. semitectum 1–V	0–salmon	Tan	0–orange	Yellow and salmon	0
F. semitectum 1–VI	0	0	0	0–orange	0
F. semitectum 1–VII	0–salmon	0–salmon	Salmon	Salmon and brown	Salmon
F. semitectum 1–VIII	0	Faint purple	0–light purple	Light purple	Purple patch
F. semitectum 1–IX	Salmon	0–salmon	Salmon	Salmon	0–salmon
F. semitectum 1–X	0–salmon	Tan	Salmon	Brown and salmon	Brown
F. semitectum 1–XI	0	0	0	0–salmon	0
F. semitectum 1–XII	0–salmon	Salmon	Salmon	Salmon	Orange
F. semitectum 1–XIII	0–salmon	0	0	Salmon and brown	0–light orange
F. semitectum 1–XIV	0–light brown	0	0–light salmon	Salmon and yellow	0–light orange

TABLE 2.—(*Continued*)

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. semitectum 1–XV	0–orange	0–salmon	Salmon	Salmon	0–light orange
F. semitectum 1–XVI	0–salmon	Salmon	Salmon	Salmon and brown	0–flesh
F. semitectum 1–XVII	0–light brown	Tan	Tan-orange	Salmon and brown	Light brown and pink
F. semitectum 1–XVIII	0–orange	Tan	Salmon	Salmon and brown	Yellowish brown
F. semitectum 2	Light salmon	Brown and salmon	Brown and salmon	Brown and salmon	0–pink
F. solani 1	0	0	0	Pink	0
F. solani 2–I	0	0	0–purple	Pink	Light purple
F. solani 2–II	0–bluish gray	0–blue-green	0–bluish gray	Faint purple	0
F. solani 3–I	Blue-green patch	Blue-green patch-faint purple	0–blue-green patch	0–reddish purple	0–blue-green
F. solani 3–II	0–blue-green patch	0	0	0	0
F. solani 3–III	0	0	0	0–faint pink	0
F. solani 4–I	0	0	0	0–faint purple	0
F. solani 4–II	0–blue-green patch	0 –blue-green and purple patch	0–blue-green patch	Flesh-pink	Trace of blue-green
F. solani minus 1–I	0	0	0	0–pink and light brown	0–tan
F. solani minus 1–II	0	0	0	0–pink	0
F. solani minus 2–I	0–blue-green	0	0–bluish green	Pink or purple	0–brown
F. solani minus 2–II	0	0	0	0	0
F. solani suffuscum	0	0–trace of blue-green	0–red	Orange-pink	0–orange
F. spinaceae 1	0	0	0	Trace of purple	0–faint purple or blue-green
F. spinaceae 2	0	0	0–trace of purple	Purplish red	0–faint purplish gray
F. striatum 1–I	0	0	0	0	0
F. striatum 1–II	Dark blue-green	Brownish purple	Brownish purple	Purple-brown	Purple and pink
F. striatum 2	Blue-green patch	0	0–blue-green	Orange	0–greenish brown
F. striatum 3–I	0–faint blue-green	Yellow	Yellow	Red-purple	Light brown
F. striatum 3–II	0	0–yellow	0–yellow	0–faint purple	0–light brown
F. sulphureum 1	0	0	0	0	0
F. sulphureum 2	0–blue-green zone	0–pink and yellow	0–yellow and orange	Yellow	Yellow-red-purple
F. sulphureum 3	0–orange	Pale orange	Orange	Salmon	Orange

TABLE 2.— (*Continued*)

Organism	Malt extract agar	Potassium nitrate agar	Peptone agar	Steamed rice	Oatmeal agar
F. theobrome	0	0	0	0	0
F. tracheiphilum	0	0	0	0	0–faint purple patch
F. trichothecioides 1	0	0	0	0–pink	0
F. trichothecioides 2–I	0	0	0	Orange and salmon	0
F. trichothecioides 2–II	0–orange	Orange	0–orange	Salmon	Orange
F. trichothecioides 2–III	0	0	0	Salmon	0
F. trichothecioides 3–I	0	0	0	0	0
F. trichothecioides 3–II	0	0	0	0	0
F. trichothecioides 4–I	0	0	0	Yellow and salmon	0
F. trichothecioides 4–II	0	0	0	0–light brown and faint purple	0–pink
F. trichothecioides 5	0	0–purple	0–purple	Blue and trace of red	Purple
F. trichothecioides 6	0	Faint purple	0–blue-green	Red	Light purple
F. trichothecioides 7–I	0	0	0	0–light purple	0
F. trichothecioides 7–II	0	0	0	0	0
F. tricinctum 1–II	Purplish red	Red and yellow	0–yellow	Orange-tan	Reddish purple
F. tricinctum 1–III	Purplish red	Red and orange	Purplish red patch	Tan-flesh	Reddish purple-yellow
F. tricinctum 1–IV	0–purplish red	Red and orange	Purplish red patch	Flesh-gold	Reddish purple-yellow
F. tricinctum 1–V	0	0–red and orange	Purplish red patch	0–tan and pink	Reddish purple-yellow
F. tricinctum 1–VI	0–red zone	Red and yellow	Red and yellow patch	Tan-faint pink	Reddish purple-yellow
F. tricinctum 1–VII	Purplish red patch	Crimson-orange and red	Crimson	Crimson-tan	Crimson
F. tricinctum 1–VIII	Purplish red	0–red	0–red patch	0–tan	Reddish purple patch
F. tricinctum 1–IX	Purplish red	Red and orange	Patch of red and blue-green	Tan-reddish purple	Reddish purple-yellow
F. tricinctum 2	0–red patch	Red-pink patch	Orange with purple patch	Orange	Red
F. uncinatum	0–orange	Yellow	0–orange and blue-green patch	Yellow	Orange–faint purple
F. urticearum I	0	0	0	0	0
F. urticearum II	0	0	0	0	0
F. vasinfectum	0	0–faint purple	0–purple	Pink	Purple
F. viride 1	0–bluish gray	0–trace of purple	0–bluish gray patch	Brownish purple-pink	0–purplish gray
F. viride 2	0	0–trace of purple	0	Trace of pink	0–purple
F. viticola	Reddish purple patch	Red-pink and orange	0	Yellow	Reddish purple and orange
F. zonatum 1	0	0	0	0	0–lines of reddish purple
F. zonatum 2	0	0	0	0	0
F. zonatum 3	0	0	0	0–faint pink	0–pink

and 1–II, *F. hyperoxysporum* 1, *F. javanicum* 2, *F. lini*, *F. loncheceras*, *F. moniliforme* 5, *F. moniliforme subglutinans*, *F. orthoceras*, *F. orthoceras triseptatum*, *F. oxysporum* 1 and 2, *F. solani* 2–II, *F. trichothecioides* 5, *F. tricinctum* 1–V and 1–VIII, and many others may at one time appear in bright colors, and then, on subsequent trials, grow without the production of the faintest pigment. Nor is this confined to the purely synthetic agar. All of the remaining media exhibit similar phenomena in a more or less striking degree. Many of these cultures have been repeated sufficiently to prove that such fluctuations are inherent in the protoplasm rather than the result of faulty technique or accidental contamination.

The different strains of the same species and the different variants of the same strain are not, necessarily, very similar in their color production. Even after the elimination of the less bright shades, such as flesh, salmon, and yellow, also such pigments as manifest themselves in patches, lines, or irregular spots, there will still be a large number of departures from the specific path. The following are examples of such cases occurring on malt-extract agar. *Fusarium acuminatum* 1 is colorless; so are 2–V, 2–VI and 2–VII; 2–I and 2–III are sometimes colorless and sometimes red, while 2–IV is always red. *F. coeruleum* 1–I and 3 are blue-green, 1–II sometimes colorless and sometimes showing blue-green, while no. 2 is always colorless. *F. culmorum* 1–I is usually bright red with golden aerial hyphae, although occasionally it may appear colorless; 1–II is usually colorless, but sometimes shows the red and gold. The remaining strains of this species show all sorts of variation, such as crimson, purplish red, orange, or, as in case of 6–I, may be always colorless. Two strains of *F. eumartii* may produce blue-green pigment, but the remaining three are always colorless. *F. fructigenum maius* 2–I has been extremely erratic; at one time it has repeatedly produced a bright red pigment, and at other times it has failed to form any color. Such periods have alternated in the life of this organism, and all attempts to control them have been futile. *F. herbarum* 1 has appeared in purple or red color, *F. herbarum* 2 has formed no pigment. Similarly, *F. herbarum giberalloides* I seems unable to produce pigment, whereas no. II has been a dazzling red with beautiful patterns along the outer edges of the colony. *F. striatum* 1–I is colorless and 1–II dark blue green, becoming black with age.

Similar variations are to be found on the other media, demonstrating that such variations are not peculiar to a given agar. Of the complex vegetable media, the steamed rice is less conducive to color differentiation and spore production than oatmeal agar. It seems that too many organisms tend to develop some shade of pigment on steamed rice and thus the use of this medium serves to reduce clear-cut differences between species and strains.

PRODUCTION OF SPORE MASSES ON DIFFERENT MEDIA

Only one other characteristic aside from color formation has been considered in connection with work on solid media, namely, the formation of spore masses. Since sclerotia have been extremely erratic and seem to form only when the organisms are freshly isolated from their hosts, no significance has been attached to their periodic appearance. The spore masses, however, have been fairly uniform. The writer has avoided the use of the terms sporodochia and pseudopionnotes because he feels that they are often confused by the average worker, especially when there are too many uncertain forms between the borderlines of these two types of fruiting structures. The spore mass is used here merely to designate any gelatinous exudation of spores which can be seen and differentiated with the unaided eye. This practice may seem a bit unorthodox, but is, nevertheless, extremely convenient.

The readings were made one month after inoculations. The temperature, in all cases, was 25° C. It is probable that if temperature, length of growing period, and the quality and quantity of the nutrient agars were altered, the results would be quite different. However, it is not the purpose of this work to make definite statements concerning the ability of these Fusaria to show positive or negative reactions under all conditions. All the results here tabulated concern themselves with the immediate environmental factors under which they were produced, and their proper interpretation should be confined to the experimental limits that are outlined here.

To conserve space, only those organisms which gave positive results have been included in the table following.

Oatmeal agar induces the formation of spore masses in more organisms than the other three, although the difference is small. The significant part, however, is not the comparative efficiency of different media in this respect, but the fact that the strains of the same species, and even the variants of the same strain, all coming from a single spore, should show such sharp differences in their ability to form spore masses. When the results obtained with oatmeal agar are examined, it can be seen that *F. bullatum* I produces no spore masses, while *F. bullatum* II does; *F. coeruleum* 1–I has spore masses and *F. coeruleum* 1–II, 2 and 3 do not; *F. filiferum* I gives negative results and *F. filiferum* II gives positive results; *F. herbarum gibberalloides* I appears without spore masses, while II appears with plenty of them; *F. incarnatum* IV does possess them, *F. incarnatum* I, II and V do not; of the three variants of *F. martii phaseoli* 1 only one has spore masses, and only *F. martii phaseoli* 2–I can produce these gelatinous guttations. Only three of the eighteen variants of *F. semitectum* 1 have them, and only two of all the forms of *F. trichothecioides* give positive results in

TABLE 3.—*Production of spore masses on different nutrient agars (1 month at 25° C.)*[6]

Organism	Oatmeal Agar Relative Quantity and Color of Spore Masses		Synthetic Agar Relative Quantity and Color of Spore Masses		Malt Extract Agar Relative Quantity and Color of Spore Masses		Peptone Agar Relative Quantity and Color of Spore Masses	
ithophilum	IV	Orange	III	Orange	0	0	0	0
illatum II	III	Orange	II	Orange	IV	Orange	II	Orange
illatum brevis	III	Orange	0	0	0	0	0	0
eruleum 1–I	V	Gray	V	Blue-green & cream	III	Bluish gray	V	Blue-green
ilmorum 4–II	IV	Brown	III	Brown	IV	Orange	IV	Brown
cemocellulare	II	Cream	0	0	II	Orange	0	0
martii 1–I	0	0	V	Blue-green	IV	Blue	III	Blue-green
martii 2–I	II	Gray	IV	Gray	IV	Blue	V	Blue-green
martii 2–II	II	Cream	II	Cream	0	0	III	Cream
martii 3	II	Cream	0	0	0	0	0	0
liferum II	IV	Orange	V	Orange	III	Orange	IV	Orange
occiferum	III	Cream	II	Gray	0	0	IV	Cream & blue-green
ructigenum maius 1	II	Orange	0	0	0	0	0	0
ructigenum maius 2–I	III	Orange	II	Orange	IV	Orange	0	0
ructigenum maius 2–II	II	Orange	III	Orange	IV	Orange	0	0
ructigenum maius 2–V	III	Orange	V	Orange	V	Orange	V	Orange
ructigenum maius 3–III	V	Brown	0	0	III	Orange	0	0
rbarum gibberalloides II	IV	Orange	III	Orange	III	Orange	III	Orange
carnatum III	0	0	V	Orange	0	0	0	0
carnatum IV	III	Orange	0	0	IV	Orange	0	0
tulatum I	V	Cream	V	Orange	V	Cream	IV	Blue
tulatum II	III	Cream	III	Cream	0	0	II	Cream
acroceras	0	0	III	Orange	V	Orange	IV	Orange
artii 1	II	Cream	II	Cream	II	Orange	0	0
artii phaseoli 1–I	IV	Blue-green	III	Blue-green	IV	Blue	III	Blue-green
artii phaseoli 2–I	III	Blue-green	IV	Blue-green	IV	Blue	IV	Blue-green
artii pisi	II	Cream	0	0	0	0	III	Cream
artii viride	0	0	0	0	0	0	III	Orange
oniliforme erumpens II	0	0	IV	Orange	0	0	V	Orange
ssicolum 1	0	0	0	0	II	Orange	0	0
ssicolum 2	IV	Cream	V	Cream	0	0	V	Cream
idicicola	III	Cream	III	Gray	0	0	III	Gray

[6] Symbol I signifies very slight; V, very abundant; the remaining symbols indicate the proportional abundance of the spore masses.

TABLE 3.—(*Continued*)

Organism	Oatmeal Agar Relative Quantity and Color of Spore Masses		Synthetic Agar Relative Quantity and Color of Spore Masses		Malt Extract Agar Relative Quantity and Color of Spore Masses		Peptone Agar Relative Quantity and Color of Spore Masses	
ambucinum	IV	Orange	V	Orange	III	Orange	IV	Orange
emitectum X	IV	Orange	II	Orange	III	Orange	0	0
emitectum XIII	0	0	0	0	II	Orange	0	0
emitectum XVII	IV	Orange	IV	Orange	IV	Orange	0	0
emitectum XVIII	III	Orange	IV	Orange	IV	Orange	II	Orange
olani 2–I	III	Blue-green & gray	0	0	II	Gray	0	0
olani 2–II	IV	Cream	V	Cream	0	0	V	Blue-green
olani 3–I	IV	Cream	III	Gray	II	Blue-green	III	Blue-green
olani minus 2–I	IV	Blue-green	V	Cream & blue-green	0	0	V	Blue-green
olani minus 2–II	0	0	0	0	III	Gray	0	0
riatum 1–II	0	0	0	0	III	Gray	0	0
riatum 2	III	Gray	IV	Gray	0	0	V	Gray
riatum 3–I	0	0	III	Cream	0	0	II	Gray
ulphureum 2	V	Yellow	V	Yellow	IV	Cream	0	0
ulphureum 3	0–V	Orange	V	Orange	III	Orange	V	Orange
richothecioides 2–II	IV	Orange	V	Orange	V	Orange	0	0
richothecioides 4–I	0	0	0	0	III	Cream	0	0
richothecioides 4–II	III	Cream	V	Light brown	III	Cream	0	0
ricinctum 1–III	III	Brown	III	Orange	III	Orange	0–V	Brown
ricinctum 1–IV	IV	Yellow and pink	III	Pink	0	0	0	0
ricinctum 1–IX	IV	Light brown	0	0	0	0	0	0
ricinctum 2	0	0	III	Cream	III	Orange	0	0
ncinatum	IV	Light brown	0	0	0	0	0	0
isinfectum	II	Gray	0	0	0	0	0	0
ride 1	III	Cream	III	Cream	III	Gray	IV	Blue-green
ticola	IV	Orange	IV	Orange	III	Orange	V	Orange
matum 2	IV	Cream	0	0	III	Cream	V	Cream
matum 3	III	Orange	IV	Cream	IV	Orange	V	Cream

this respect. The other agars show similar and just as striking differences, and prove that these are not accidental fluctuations or contaminations but specific behavior of the individual organism regardless of its artificial affinities with sister forms.

REPRODUCTION ON MALT EXTRACT AGAR

Reproduction in Fusaria is not as dependable a criterion as other vital activities but it exhibits certain interesting modifications which may exert some influence in a proper construction of species concept. It is the chief purpose of this paper to demonstrate that the strains of the same species may often exhibit as wide variations among themselves as do the different species; therefore, even though the reproduction factor should not be given a primary rôle in a scheme of differentiation, it should, nevertheless, serve a useful purpose.

All microscopic examinations were begun one month after the transfers were made to malt-extract agar. The work was repeated at least once and sometimes twice, and it was extended over one year, so that a long period elapsed between the two trials. After the notes on the first experiment were taken, they were filed away and no reference was made to them until after the second set of notes was taken. This practice has been followed throughout the work and has been found very useful in reducing the error due to the personal equation and unconscious point-stretching to an absolute minimum. Each reading, expressed in Roman numerals, is separated from others by a dash, such as II–0–III; this means that one set of cultures yielded macroconidia the approximate amount of which was estimated as II; another set of cultures was read as 0, and another as III. This practice has been followed throughout the work.

Even a casual glance at table 4 is sufficient to show the numerous and striking variations exhibited by the different strains of the same species. *F. acuminatum* 2–IV, 2–VI, and 2–VII produce no macroconidia, while the remaining six do. *F. coeruleum* 1–I is the only strain of this species capable of forming macroconidia on malt-extract agar and number 3 is the only one unable to form microconidia. *F. culmorum* 6–I and 6–II, and perhaps number 2, form microconidia, the remaining strains do not. All except *F. eumartii* 1–I give rise to microconidia. *F. fructigenum maius* 1 and 2–VIII and 3–II produce chlamydospores but the others do not. *F. herbarum* 1 seems to be sterile, while number 2 forms both micro- and macroconidia. *F. hyperoxysporum* 1 possesses chlamydospores, while number 2 has no such spores. *F. lateritium* I has an abundance of macroconidia, but number II is sterile. *F. martii* 3–I and 3–II have no macroconidia, while numbers 1 and 2 may produce them. *F. martii phaseoli* 1–II is the only strain of this species capable of giving rise to microconidia, 1–I and 2–I are the only ones

TABLE 4.—*Reproduction on malt-extract agar (one month at 25° C.)*[7]

Organism	Microconidia	Macroconidia	Chlamydospores
F. acuminatum 1	II–0–0	II–0–V	V–V–V
F. acuminatum 2–I	0–0	IV–I	III–IV
F. acuminatum 2–II	0–0–0	0–III–I	IV–IV–V
F. acuminatum 2–III	0–0–0	0–IV–0	0–III–V
F. acuminatum 2–IV	0–0	0–0	II–III
F. acuminatum 2–V	0–I–0	0–I–0	0–III–0
F. acuminatum 2–VI	0–0	0–0	0–I
F. acuminatum 2–VII	0–0	0–0	0–I
F. acuminatum 2–VIII	0–0–0	II–III–III	0–III–II
F. alluviale	III–III	0–I	V–IV
F. anthophilum	I–0	V–IV	0–0
F. aquaeductuum pusilum	II–IV	III–IV	V–IV
F. aquaeductuum volutum	0–0	IV–IV	0–0
F. arcuatum	I–0–I	III–III–II	0–III–I
F. asclerotium	V–V–V	III–0–0	IV–V–V
F. aurantiacum	V–IV	0–I	V–IV
F. batatatis 1	III–III	II–II	V–V
F. batatatis 2	V–V	III–II	V–IV
F. bulbigenum	IV–IV	III–I	V–V
F. bullatum I	II–II	III–V	0–0
F. bullatum II	I–IV	V–IV	0–IV
F. bullatum brevius	II–0–III	V–IV–0	0–0–II
F. camptoceras I	II–II	IV–V	0–0
F. camptoceras II	II–II	IV–V	0–0
F. caudatum	III–0–II	III–III–III	III–III–III
F. chlamydosporum	0–I	0–I	V–V
F. chlamydosporum II	0–0–III–0	0–I–0–0	V–V–V–IV
F. chlamydosporum III	II–III	0–0	V–V
F. chenopodinum	III–III	II–IV	0–0
F. coeruleum 1–I	IV–IV	III–IV	IV–IV
F. coeruleum 1–II	III–II	0–0	III–III
F. coeruleum 2	IV–III–III	0–0–0	0–II–III
F. coeruleum 3	0–0	0–0	II–III
F. commutatum	III–III	0–0	0–0
F. conglutinans	III–III–II	I–0–0	II–0–IV
F. cubense 1	V–IV	III–III	V–IV
F. cubense 2	V–III	0–0	IV–IV
F. culmorum 1–I	0–0	III–II	0–0
F. culmorum 1–II	0–0	II–II	0–0
F. culmorum 2	I–0	V–V	0–0
F. culmorum 3–I	0–0	IV–III	IV–III
F. culmorum 3–II	0–0	III–IV	II–III
F. culmorum 4–I	0–0	IV–IV	IV–III
F. culmorum 4–II	0–0	V–V	IV–IV
F. culmorum 5	0–0	IV–III	IV–V
F. culmorum 6–I	IV–V–IV	0–II–0	IV–IV–IV
F. culmorum 6–II	I–I	III–III	0–0
F. decemcellulare	III–III–IV	0–V–0–V	0–0–0
F. dimerum	IV–V	0–0	IV–III
F. diversisporum	0–I–II	III–IV–IV	III–0–0
F. ensiforme	0–IV–IV	0–0–0	II–II–I
F. eumartii 1–I	0–0–0	V–V–V	0–II–0
F. eumartii 1–II	IV–0–IV	0–II–0	V–II–V
F. eumartii 2–I	III–III	IV–IV	0–0

[7] 0 signifies total absence of spores; I, very few; II, few; III, fair; IV, abundant; V, very abundant.

TABLE 4.—(*Continued*)

Organism	Microconidia	Macroconidia	Chlamydospores
F. eumartii 2–II	IV–II	III–III	II–IV
F. eumartii 3	IV–III	III–III	0–0
F. euoxysporum	III–II–III	III–0–I	V–V–V
F. falcatum	II–0–0–I	II–0–0–II	II–III–IV–III
F. filiferum I	I–0	I–I	V–V
F. filiferum II	0–0–0	V–V–V	IV–III–V
F. flocciferum	0–I–II	0–V–V	II–IV–V
F. fructigenum	0–0–0	0–0–0	0–0–0
F. fructigenum maius 1	0–0–II	III–III–III	III–0–III
F. fructigenum maius 2–I	0–0	V–V	0–0
F. fructigenum maius 2–II	0–0	V–V	0–0
F. fructigenum maius 2–III	0–0–0	II–0–II	0–0–0
F. fructigenum maius 2–IV	0–0–0	0–III–0	0–0–0
F. fructigenum maius 2–V	0–0	V–V	0–0
F. fructigenum maius 2–VII	0–I–0	0–II–0	0–0–0
F. fructigenum maius 2–VIII	0–I–0	0–I–0	0–I–0
F. fructigenum maius 3–I	0–0	0–0	0–0
F. fructigenum maius 3–II	IV–0–0	0–0–V	II–0–0
F. fructigenum maius 3–III	0–0	V–V	0–0
F. graminum 1	0–V–0	0–II–0	III–0–0
F. graminum 2–I	0–I	I–0	II–II
F. graminum 2–II	III–III	III–III	0–III
F. gibbosum	0–I–0	0–0–III	III–IV–V
F. herbarum 1	0–0–0	0–0–0	0–0–0
F. herbarum 2	II–0–II	II–0–II	0–0–0
F. herbarum gibberalloides I	IV–I–III	IV–IV–V	0–0–0
F. herbarum gibberalloides II	0–II–I	V–IV–V	0–0–0
F. herbarum pirinum I	0–0	III–II	0–0
F. herbarum pirinum II	0–0	0–0	0–0
F. hyperoxysporum 1	V–V–V	0–II–II	V–V–V
F. hyperoxysporum 2	V–V	III–III	0–0
F. incarnatum I	I–II	V–V	0–0
F. incarnatum II	0–II–0	0–III–I	0–0–0
F. incarnatum IV	II–II	IV–V	0–0
F. incarnatum V	II–II	IV–V	0–0
F. javanicum 1	III–III	III–II	V–V
F. javanicum 2	IV–III	I–0	V–V
F. lateritium I	0–0	IV–IV	0–0
F. lateritium II	0–0	0–0	0–0
F. lini	V–IV–V	III–0–0	IV–IV–V
F. lolii	0–0	III–III	0–0
F. loncheceras	V–IV	0–0	V–IV
F. loncheceras microsporum	III–II–V	0–I–0	II–III–III
F. longipes	I–IV	0–0	V–IV
F. lutulatum I	III–II	V–V	IV–III
F. lutulatum II	III–I	V–V	III–III
F. lycopersici 1	II–II	II–II	IV–II
F. lycopersici 2	IV–III	III–II	V–V
F. malli	V–IV–V	0–II–III	III–IV–V
F. macroceras	0–0	V–V	0–0
F. martii 1	V–IV–V	III–0–II	IV–IV–IV
F. martii 2	V–V	0–I	V–IV
F. martii 3–I	IV–III	0–0	IV–IV
F. martii 3–II	IV–III	0–0	IV–III
F. martii minus	IV–III	II–III	V–II
F. martii phaseoli 1–I	0–0–0	V–V–IV	0–II–III
F. martii phaseoli 1–II	V–0–V	0–0–0	V–II–V

TABLE 4.—(*Continued*)

Organism	Microconidia	Macroconidia	Chlamydospores
F. martii phaseoli 1–III	0–0–0	0–0–0	0–0–0
F. martii phaseoli 2–I	0–0	V–V	II–II
F. martii phaseoli 2–II	0–0	0–0	II–II
F. martii pisi	IV–IV	IV–II	IV–IV
F. martii viride	V–III–III	V–0–V	III–IV–V
F. moniliforme 1	V–V	0–I	0–0
F. moniliforme 2	V–V–V	0–III–II	0–0–0
F. moniliforme 3	IV–III	0–0	0–0
F. moniliforme 4	IV–III–III	0–II–0	0–0–0
F. moniliforme 5	III–III	0–I	0–0
F. moniliforme erumpens I	III–IV	V–III	0–0
F. moniliforme erumpens II	V–IV	IV–III	0–0
F. moniliforme minus	IV–IV	III–III	0–0
F. moniliforme subglutinans	V–II	IV–V	0–0
F. neoceras	II–IV	V–V	0–0
F. niveum 1	IV–IV	0–I	V–V
F. niveum 2	III–0–III	0–0–0	IV–V–IV
F. orthoceras	IV–III	0–I	V–IV
F. orthoceras triseptatum	V–IV–IV	0–II–II	IV–III–IV
F. ossicolum 1	0–IV–0	II–III–I	V–IV–V
F. ossicolum 2	III–0–I	V–V–V	V–0–III
F. oxysporum 1	II–III–III	IV–0–II	IV–V–V
F. oxysporum 2	V–IV–V	0–II–0	V–IV–V
F. oxysporum nicotianae	V–V	0–I	V–IV
F. paspalicola	0–0	II–IV	0–0
F. poae I	II–I	0–0	0–0
F. poae II	IV–IV	III–III	0–0
F. pirinum	II–0–0	III–0–II	0–0–0
F. polymorphum	0–0	III–III	II–V
F. pusillum	III–IV	IV–V	V–III
F. radicicola	V–IV–III	III–0–III	V–V–V
F. rhizochromatistes	V–IV	0–0	V–IV
F. rhizochromatistes-microsclerotium	III–IV	0–0	V–IV
F. sambucinum 1	I–II	V–V	0–0
F. sambucinum 2	IV–IV	0–0	IV–III
F. scirpi	0–0–0	0–0–0	0–III–III
F. sclerostromaton	IV–IV	III–II	IV–V
F. semitectum 1–I	IV–III–III	IV–V–V	0–II–0
F. semitectum 1–II	0–IV–V	0–III–0	0–II–III
F. semitectum 1–III	III–III–III	III–IV–IV	0–II–0
F. semitectum 1–IV	0–0–0	0–0–0	0–III–0
F. semitectum 1–V	0–0–0	0–0–0	0–II–0
F. semitectum 1–VI	0–0	0–0	0–0
F. semitectum 1–VII	0–0	0–0	0–III
F. semitectum 1–VIII	IV–V	III–II	IV–IV
F. semitectum 1–IX	0–0	0–0	0–0
F. semitectum 1–X	0–0	V–V	0–0
F. semitectum 1–XI	0–0	IV–IV	IV–IV
F. semitectum 1–XII	0–I	II–I	0–0
F. semitectum 1–XIII	0–0	II–III	0–0
F. semitectum 1–XIV	0–0	0–0	0–0
F. semitectum 1–XV	0–0	0–0	0–0
F. semitectum 1–XVI	0–0	0–0	0–0
F. semitectum 1–XVII	IV–III	IV–V	0–0
F. semitectum 1–XVIII	II–I	V–V	0–0
F. semitectum 2	II–0–II	III–III–III	IV–0–IV

TABLE 4.—(*Continued*)

Organism	Microconidia	Macroconidia	Chlamydospores
F. solani 1	IV–0–III	0–III–II	IV–V–IV
F. solani 2–I	V–V–V	0–II–0	IV–III–V
F. solani 2–II	II–IV	V–IV	III–IV
F. solani 3–I	II–III	V–V	IV–IV
F. solani 3–II	III–III	0–0	V–III
F. solani 3–III	IV–III	III–III	V–IV
F. solani 4–I	0–0	0–0	IV–III
F. solani 4–II	III–III	III–V	III–III
F. solani minus 1–I	IV–IV–IV	0–II–I	IV–IV–IV
F. solani minus 1–II	IV–IV	0–0	IV–IV
F. solani minus 2–I	III–III	IV–IV	III–III
F. solani minus 2–II	IV–IV–III	0–II–0	V–IV–II
F. solani suffuscum	II–II	0–0	V–V
F. spinaceae 1	V–IV	III–III	V–IV
F. spinaceae 2	V–IV	III–III	IV–IV
F. striatum 1–I	V–IV	0–0	V–IV
F. striatum 1–II	V–IV	0–0	II–IV
F. striatum 2	IV–III	IV–IV	IV–III
F. striatum 3–I	III–V–0	IV–II–0	V–V–0
F. striatum 3–II	IV–IV	IV–III	IV–III
F. sulphureum 1	0–0	III–II	0–0
F. sulphureum 2	0–0	V–V	0–0
F. sulphureum 3	0–0	V–V	0–0
F. theobrome	IV–III	0–0	IV–III
F. tracheiphilum	V–IV–V	II–III–II	0–III–II
F. trichothecioides 1	III–0–II	0–IV–0	0–0–0
F. trichothecioides 2–I	0–0	I–IV	0–0
F. trichothecioides 2–II	0–0	V–V	0–0
F. trichothecioides 2–III	0–0–0	II–0–III	0–0–0
F. trichothecioides 3–I	0–0–0	0–V–II	V–V–V
F. trichothecioides 3–II	I–II	II–III	0–0
F. trichothecioides 4–I	0–I–II	0–V–II	0–0–0
F. trichothecioides 4–II	I–0–0	IV–IV–III	IV–0–V
F. trichothecioides 5	III–0	0–0	IV–V
F. trichothecioides 6	V–IV	0–II	V–IV
F. trichothecioides 7–I	0–0	0–0	IV–IV
F. trichothecioides 7–II	IV–IV	III–II	0–0
F. tricinctum 1–II	II–IV–IV	II–V–III	0–0–0
F. tricinctum 1–III	III–IV–IV	IV–V–V	0–0–0
F. tricinctum 1–IV	III–IV–IV	IV–V–IV	0–0–0
F. tricinctum 1–V	III–IV–IV	IV–V–IV	0–0–0
F. tricinctum 1–VI	V–IV–V	III–IV–III	0–0–0
F. tricinctum 1–VII	II–IV–IV	II–V–III	0–0–0
F. tricinctum 1–VIII	0–IV–III	0–V–II	0–0–0
F. tricinctum 1–IX	III–IV–IV	IV–V–IV	0–0–0
F. tricinctum 2	II–0–II	IV–V–IV	0–0–0
F. uncinatum	IV–IV–IV	III–III–III	II–0–I
F. urticearum I	0–0	V–V	0–0
F. urticearum II	0–0	I–0	0–0
F. vasinfectum	I–0–II	IV–III–III	IV–IV–IV
F. viride 1	V–III–III	V–0–V	III–V–IV
F. viride 2	IV–V	II–I	V–V
F. viticola	III–0–0	IV–IV–V	0–0–0
F. zonatum 1	IV–II	0–0	IV–IV
F. zonatum 2	III–IV	V–III	II–V
F. zonatum 3	IV–III	IV–V	V–IV

possessing macroconidia, and 1–III is the only one which has no chlamydospores; in fact, it forms no spores at all. *F. moniliforme* 3 is devoid of macroconidia, and number 5 produces such bodies only sparsely and occasionally. *F. poae* II has macroconidia, *F. poae* I does not. *F. semitectum* 1–VI, 1–IX, 1–XIV, 1–XV, and 1–XVI are sterile; 1–XII, 1–XIII, 1–XVII, and 1–XVIII have no chlamydospores; only 1–VIII, 1–XII, 1–XVII, 1–XVIII, and number 2 form microconidia, and only 1–VIII, 1–X, 1–XI, 1–XII, 1–XIII, 1–XVII, 1–XVIII, and number 2 form macroconidia. *F. solani* 3–II produces no macroconidia, while 4–I produces neither micronor macroconidia. *F. striatum* 1–I and 1–II do not produce macroconidia, while number 2, 3–I and 3–II do. Only five of the twelve strains of *F. trichothecioides* yield chlamydospores, seven form microconidia and only two fail to give rise to macroconidia. *F. zonatum* is the only strain of the three members of this species which fails in the production of macroconidia. These are just a few examples. Similar results can be seen throughout the table. In addition, there is quite an inconsistency of results. At one time a culture may give rise to a large quantity of spores; at another, not a single spore can be found. No reaction seems to be free of such inconsistencies.

THE EFFECT OF TEMPERATURE ON GROWTH

Three temperatures, 5° C., 34° C., and 37.5° C., have been used in this experiment. Solid agar was found unsatisfactory because, in the first place, it adsorbs too much of the moisture and the inoculum cannot establish a rapid and efficient contact when the temperatures are so extreme. In the second place, the rapid drying of agar introduces unfavorable factors and leads the investigator to erroneous conclusions. A nutrient solution, prepared according to the formula given for malt-extract medium, but with the agar eliminated, was tried and found satisfactory. Since food concentration is not an inhibiting factor in the growth of Fusaria, evaporation from the solution under the high temperatures cannot be considered harmful.

Five c.c. of nutrient solution[8] was poured into each test-tube and sterilized. Immediately after the transfers were made the cultures were placed in the constant-temperature chambers and two weeks later the readings were made. The work was repeated at least once. The results are shown in table 5.

It can readily be seen that fluctuating reactions occur fairly abundantly even under such a standard environmental factor as temperature. Wher-

[8] Dry malt extract 10 g., dextrose 10 g., potassium nitrate 2 g., dihydrogen potassium phosphate 1 g., magnesium sulphate 1 g., distilled water 1,000 c.c. This solution was also used in experiments with tartaric acid, while in case of all the subsequent work it was somewhat modified by the elimination of malt extract.

TABLE 5.—*The effect of temperature on growth (two weeks at each temperature)*[9]

Organism	5° C.	34° C.	37.5° C.
F. acuminatum 1	0–I–0–0	IV–IV–IV	II–II
F. acuminatum 2–1	II–II–III	II–I	0–0–0
F. acuminatum 2–II	II–II–II	I–0	0–0–0
F. acuminatum 2–III	II–III–III	I–I	0–0–0
F. acuminatum 2–IV	II–III–III	I–0–I	0–0–0
F. acuminatum 2–V	I–II–I	0–0	0–0–0
F. acuminatum 2–VI	II–II–II	0–0	0–0–0
F. acuminatum 2–VII	III–III–III	II–I	0–0–0
F. acuminatum 2–VIII	II–III–III	I–0–II	0–0–0
F. alluviale	0–0	III–III	II–II
F. anthophilum	IV–V–IV	III–I	0–0–0
F. aquaeductuum pusilum	II–II–II	0–0	0–0
F. aquaeductuum volutum	II–III–III	0–0	0–0–0
F. arcuatum	III–IV–III	0–0	0–0–0
F. asclerotium	I–I–0	II–IV	0–I–I
F. aurantiacum	0–0–0	II–III	0–0
F. batatatis 1	0–0–0	V–V	II–II
F. batatatis 2	0–I–0	II–III	0–0
F. bulbigenum	I–I	IV–III	0–I–I
F. bullatum I	0–0–I–I	III–III	0–0
F. bullatum II	I–I–0	III–III	0–0–I–0
F. bullatum brevius	I–I	IV–V	0–0
F. camptoceras I	I–I–I	II–II–II	0–0–0
F. camptoceras II	I–I–I	II–II	0–0–0
F. caudatum	I–I–I	IV–IV	0–0
F. chlamydosporum I	I–0–0–0	V–V	III–I–II
F. chlamydosporum II	0–0–I–0	IV–IV	III–I–II
F. chlamydosporum III	0–0–0–0	V–V	III–I–II
F. chenopodinum	II–II–II	I–0–II	0–0–0
F. coeruleum 1–I	II–II–II–I	III–IV	I–0–I
F. coeruleum 1–II	I–0–I	III–V	0–0–0
F. coeruleum 2	I–I	IV–V	0–0
F. coeruleum 3	II–II	0–0	0–0–0
F. commutatum	III–IV–IV	II–II	0–0–0
F. conglutinans	0–0	I–I–II	0–0
F. cubense 1	0–0–0	III–III	0–0
F. cubense 2	0–0–0	III–V	0–0
F. culmorum 1–I	III–III	0–0	0–0–0
F. culmorum 1–II	II–III	I–I	0–0–0
F. culmorum 2	II–III–II	I–0–II–III	0–0–0
F. culmorum 3–I	I–II–IV	II–II	0–0–0
F. culmorum 3–II	I–II–II	II–II	0–0–0
F. culmorum 4–I	II–III–III	II–II	0–0–0
F. culmorum 4–II	II–III–IV	II–II	0–0–0
F. culmorum 5	III–II–I	II–II	0–0–0
F. culmorum 6–I	0–I–0	II–III	0–I–0–I–I
F. culmorum 6–II	II–II–II	II–II	0–0–0
F. decemcellulare	0–0–0	II–III	I–I–I
F. dimerum	0–0–0	IV–IV	III–III
F. diversisporum	I–0–I	III–V	0–0–0
F. ensiforme	0–0–0	III–III	0–0
F. eumartii 1–I	0–I–0	II–II	0–0–0
F. eumartii 1–II	0–0–0	I–III–IV	0–0–0
F. eumartii 2–I	II–II–III	0–0	0–0–0
F. eumartii 2–II	I–II–I	0–0	0–0–0

[9] 0 signifies no growth; I, very slight; II, slight; III, fair; IV, good; V, abundant.

TABLE 5.—(*Continued*)

Organism	5° C.	34° C.	37.5° C.
F. eumartii 3	I–II–I	II–I	0–0
F. euoxysporum	I–II–II	IV–IV	0–0
F. falcatum	0–0–I	II–II	0–0
F. filiferum I	I–I	0–I–I–II	0–0–0
F. filiferum II	II–II	0–I–II	0–0–0
F. flocciferum	II–II–IV	II–II	0–0–0
F. fructigenum	II–II–III	0–0	0–0–0
F. fructigenum maius 1	I–I–I	II–III	0–0
F. fructigenum maius 2–I	0–0–0	II–II	0–0
F. fructigenum maius 2–II	0–0–I–0	III–IV	0–0
F. fructigenum maius 2–III	0–0–I–0	II–III	0–0
F. fructigenum maius 2–IV	0–0	II–III	0–0
F. fructigenum maius 2–V	0–0–I	III–II	0–0–0
F. fructigenum maius 2–VII	0–0–I–0	I–0–II–II	0–0–0
F. fructigenum maius 2–VIII	0–0–I–0	II–III	0–0–0
F. fructigenum maius 3–I	II–III–0–II	0–0	0–0–0
F. fructigenum maius 3–II	II–III–II	0–0	0–0–0
F. fructigenum maius 3–III	II–III–III	0–0	0–0–0
F. graminum 1	II–II–III	II–I	0–0
F. graminum 2–I	0–0	IV–V	0–0
F. graminum 2–II	I–II–I–I	III–IV	0–0–0
F. gemmiperda	II–II–II	I–II	0–0
F. gibbosum	II–II–II	II–II	0–0
F. herbarum 1	II–III–II	0–0	0–0–0
F. herbarum 2	III–III–IV	0–0	0–0–0
F. herbarum gibberalloides I	II–II–III	0–0	0–0–0
F. herbarum gibberalloides II	III–III–IV	0–0	0–0–0
F. herbarum pirinum I	II–III–II	0–0	0–0–0
F. herbarum pirinum II	II–II–II	0–0	0–0
F. hyperoxysporum 1	0–I	IV–V	0–0
F. hyperoxysporum 2	0–0	II–III	0–I–0
F. incarnatum 1	0–I–0	V–V	0–0–0
F. incarnatum II	0–0	III–IV	0–0–0
F. incarnatum IV	I–I–0	IV–IV	0–0–0
F. incarnatum V	0–I–0	V–V	0–0–0
F. javanicum 1	0–0–0	IV–IV	II–III
F. javanicum 2	0–I–I	II–III	0–I–I
F. lateritium I	III–II–II	0–0	0–0–0
F. lateritium II	III–II–III	0–0	0–0–0
F. limi	I–I–I	IV–IV	0–0
F. lolii	II–III–II	III–II	0–0–0
F. loncheceras	0–I–I	II–III	0–0
F. loncheceras microsporum	0–I–I	III–II	0–0
F. longipes	0–0–0	V–II	I–I
F. lutulatum I	0–0–0	III–III	0–I–I–I
F. lutulatum II	0–0–0	III–III	0–I–I–I
F. lycopersici 1	0–0–0	IV–III	0–0
F. lycopersici 2	I–0–0	III–II	0–I
F. malli	I–I	V–IV	I–II–II
F. macroceras	II–II–II	II–II	0–0
F. martii 1	0–0–0	III–IV	II–II
F. martii 2	0–I–I	IV–IV	0–I–I
F. martii 3–I	0–0–0	III–IV	0–0–0
F. martii 3–II	0–0–0	III–IV	0–0–0
F. martii minus	0–0–0	IV–V	II–I
F. martii phaseoli 1–I	0–0–0	II–II	0–0–0

TABLE 5.—(*Continued*)

Organism	5° C.	34° C.	37.5° C.
F. martii phaseoli 1–II	0–0–0	III–IV	0–0–0
F. martii phaseoli 1–III	0–0–0	II–II	0–0–0
F. martii phaseoli 2–I	III–II–0	II–II	0–0–0
F. martii phaseoli 2–II	0–0	II–II	0–0–0
F. martii pisi	0–0–0	II–II	0–I–I
F. martii viride	0–0–0	IV–V	III–III
F. moniliforme 1	I–0–0	IV–IV	II–II
F. moniliforme 2	I–I–0	III–III	0–0
F. moniliforme 3	II–II–II	0–0–0–0	0–0–0
F. moniliforme 4	II–II–0	IV–IV	I–I
F. moniliforme 5	II–II–I	III–IV	0–I–I
F. moniliforme erumpens I	I–I	V–IV	0–I–0–I
F. moniliforme erumpens II	I–I–I	IV–IV	0–I–II
F. moniliforme minus	I–0–0	IV–V	I–II
F. moniliforme subglutinans	I–I–I	IV–V	I–II
F. neoceras	I–II–II	III–II	0–0–0
F. niveum 1	0–II–0	III–IV	0–0
F. niveum 2	I–II–0	III–IV	0–0
F. orthoceras	I–I	II–III	0–0
F. orthoceras triseptatum	I–I	II–III	0–0
F. ossicolum 1	0–I–0	III–III	0–0–0
F. ossicolum 2	III–0–III	III–III	0–0–0
F. oxysporum 1	0–I	III–IV	0–0
F. oxysporum 2	I–II	III–IV	0–I–I
F. oxysporum nicotianae	I–I	III–III	0–0
F. paspalicola	II–II–I	II–II	0–0
F. pirinum	III–III–II	II–II	0–0
F. poae I	III–III–II	II–II	0–0–0
F. poae II	III–IV–IV	III–III	0–0–0
F. polymorphum	III–III–III	II–II	0–0
F. pusillum	0–0–0	IV–V	II–II
F. radicicola	0–0–0	IV–IV	III–III
F. rhizochromatistes	I–I–I	I–II–III	0–I
F. rhizochromatistes-microsclerotium	I–I–I	II–II	0–0
F. sambucinum 1	III–III–IV	II–II	0–I
F. sambucinum 2	II–III–IV	III–II	0–0
F. scirpi	II–II–II	0–I–I	0–0
F. sclerostromaton	I–I–I	III–IV	0–0
F. semitectum 1–I	0–I–0	II–II	0–0–0
F. semitectum 1–II	0–0–0	II–II	0–0–0
F. semitectum 1–III	0–I–I	III–III	0–0–0
F. semitectum 1–IV	0–I–I	II–III	0–0–0
F. semitectum 1–V	0–0–0	II–I	0–0–0
F. semitectum 1–VI	0–0–0	II–II	0–0–0
F. semitectum 1–VII	0–I–I–I	III–III	0–0–0
F. semitectum 1–VIII	0–I–0	II–III	0–0
F. semitectum 1–IX	0–I–I	II–II	0–0
F. semitectum 1–X	0–I–I	III–III	0–0
F. semitectum 1–XI	0–I–0	III–III	0–0
F. semitectum 1–XII	0–0–I	II–II	0–0
F. semitectum 1–XIII	0–I	II–IV	0–0
F. semitectum 1–XIV	0–I–0–I	II–I	0–0
F. semitectum 1–XV	0–0–I	II–II	0–0
F. semitectum 1–XVI	0–I–0	II–III	0–0
F. semitectum 1–XVII	0–I–I	IV–IV	0–0
F. semitectum 1–XVIII	I–I	III–III	0–0

TABLE 5.—(*Continued*)

Organism	5° C.	34° C.	37.5° C.
F. semitectum 2	0–I–I	IV–IV	0–0
F. solani 1	0–0–0	V–V	III–IV
F. solani 2–I	I–0–I	II–III	0–I–II–I
F. solani 2–II	I–II–II	V–V	II–III
F. solani 3–I	0–I–0	IV–V	III–III–IV
F. solani 3–II	0–0–0	IV–V	III–III–IV
F. solani 3–III	0–II–0	IV–V	III–III–IV
F. solani 4–I	0–II–0–0	IV–V	II–II–II
F. solani 4–II	I–II–0	IV–V–V	III–IV
F. solani minus 1–I	0–0–0	V–V	II–II–II
F. solani minus 1–II	0–0–0	III–IV	III–I
F. solani minus 2–I	0–0–0	IV–IV	III–III–IV
F. solani minus 2–II	0–0	III–IV	III–III–III
F. solani suffuscum	0–0–0	III–V	0–0–0
F. spinaceae 1	I–I	0–III–III	I–I–0
F. spinaceae 2	II–I–II	II–II	0–0
F. striatum 1–I	0–0–0	IV–V	II–II–II
F. striatum 1–II	0–0–0	IV–V	II–II–II
F. striatum 2	0–0–0	V–V	II–II
F. striatum 3–I	0–0	IV–V	III–III–II
F. striatum 3–II	0–I–0–0	V–IV	III–III–IV
F. sulphureum 1	III–III–0	0–0	0–0–0
F. sulphureum 2	III–IV–V	II–II	0–0
F. sulphureum 3	III–III–III	0–0	0–0
F. theobrome	0–0–0	III–V	0–0
F. trichothecioides 1	I–I–I	III–IV	0–I–II
F. trichothecioides 2–I	II–II–III	0–0	0–0–0
F. trichothecioides 2–II	III–IV–III	0–0	0–0–0
F. trichothecioides 2–III	III–III–III	0–0	0–0–0
F. trichothecioides 3–I	II–III–II	II–II	0–0–0
F. trichothecioides 3–II	II–III–III	0–0	0–0–0
F. trichothecioides 4–I	I–I	IV–III	0–I–I–I
F. trichothecioides 4–II	II–II	I–II	0–0–0
F. trichothecioides 5	I–0–I	II–III	0–0
F. trichothecioides 6	I–I–II	II–III	II–II–II
F. trichothecioides 7–I	0–0–0	V–V	0–0–0
F. trichothecioides 7–II	II–II–II	0–0	0–0–0
F. tricinctum 1–II	II–II–III	0–0	0–0–0
F. tricinctum 1–III	II–II–III	0–0	0–0–0
F. tricinctum 1–IV	II–II–III	0–0	0–0–0
F. tricinctum 1–V	II–II–III	0–0–I	0–0–0
F. tricinctum 1–VI	II–II–III	0–0	0–0–0
F. tricinctum 1–VII	II–II–I	0–0	0–0–0
F. tricinctum 1–VIII	II–II–III	0–0	0–0–0
F. tricinctum 1–IX	II–II–II	0–0	0–0–0
F. tricinctum 2	II–III–I	0–I–I	0–0
F. uncinatum	0–I–I–II	III–III	0–0–0
F. urticearum I	IV–IV–IV	0–0	0–0–0
F. urticearum II	II–III–III	0–0–0	0–0
F. vasinfectum	I–0–0	III–III	0–0
F. viride 1	0–0–0	III–IV	III–III
F. viride 2	0–0–0	III–IV	0–I–I
F. viticola	IV–IV–V	0–0	0–0
F. zonatum 1	I–II–II	III–IV	0–0
F. zonatum 2	I–I–I	II–III	0–0
F. zonatum 3	I–I–I	II–III	0–0

ever the reaction is indicated as 0, it does not, necessarily, mean that the inoculum was killed by the adverse temperature. Sometimes it was dead after exposure to 37.5° C. Sometimes it was not. All cultures failing to show any growth at this high temperature within two weeks were transferred to a chamber maintained at a constant temperature of 25° C. and were kept there for an additional two weeks. When examined at the end of this period, it was observed that some showed a vigorous growth, while others failed to manifest any sign of life. At first it was believed that this would give a very good differentiation; but, when the experiment was repeated and the cultures were transferred from the high to the low temperature, it was seen that many of the organisms, which in a previous attempt showed no sign of life, started to make a vigorous growth. For example, *F. aquaeductuum pusilum, F. chenopodinum, F. coeruleum* 3, *F. culmorum* 1–I and II, *F. eumartii* 1–II, *F. fructigenum,* all the variants of *F. fructigenum maius* 2, *F. herbarum* 2, *F. herbarum gibberalloides* I and II, *F. lini, F. martii phaseoli* 1–I, II, and III, *F. neoceras, F. ossicolum* 2, *F. paspalicola, F. sambucinum, F. semitectum* 1–II, IV, V, and VII, *F. solani suffuscum, F. sulphureum* 1, *F. trichothecioides* 1 and 3–III, all the variants of *F. tricinctum* 1 except IV, and many others were found dead after two weeks at 37.5°. When, however, the work was repeated once or twice, it was found that, although there was no growth while the cultures were kept at 37.5° C., they started growing vigorously shortly after transfer to the lower temperature. It might be assumed that slight temperature variations would suffice to break the balance, but duplicate cultures kept under identical conditions showed totally different results. One would be dead, while the other had retained its vitality notwithstanding the fact that the inoculum consisted of two pieces of mycelium of the same age and size and both pieces were removed from the same region in the colony.

The reactions of different strains of the same species toward temperature are by no means uniform. Numerous examples can be given, but the following are sufficiently representative:

F. acuminatum 1 grows at 37.5° C., while all the strains of *F. acuminatum* 2 do not.

F. batatatis 1 tolerates the high temperature; no. 2 fails to do so.

F. coeruleum 3 is the only strain of this species unable to grow at 34° C.

F. culmorum 1–I is the only strain of this species incapable of growth at 34° C., just as *F. culmorum* 6–I is the only one showing any aversion to very low temperatures.

F. eumartii 2–I and 2–II do not grow at 34° C., while the other strains of this species are not inhibited by this temperature.

F. fructigenum maius 3–I, 3–II, and 3–III do not grow at 34° C., while no. 1 and all the mutants of no. 2 grow at this temperature.

F. graminum 2–I is inhibited by the low temperature, while 2–II and no. 1 are not.

F. moniliforme 3 is the only strain of this species unable to grow at 34° C.

F. sulphureum 2 tolerates 34° C., while nos. 1 and 3 do not.

F. trichothecioides 1, 4–I, and 7–I are the only ones among the twelve strains of this fungus capable of making some growth at 37.5° C., and nos. 2–I, 2–II, 2–III, 3–II, and 7–II are the only ones unable to grow at 34° C.

Most of the organisms which can grow at 5° C. can not tolerate 37.5° C.; and most of those that grow at the high temperature are inhibited by the low. However, there are some exceptions and a generalization is not at all permissible.

Some remarkably uniform reactions toward temperature are to be noted among the strains of various species. Such instances indicate the possibility of developing more comprehensive and more widely inclusive tests whereby the sphere of individual variations can be more readily determined and the proper segregation of species accomplished on a more dependable basis.

Growth in presence of various concentrations of tartaric acid

Nine different concentrations of tartaric acid were used in this experiment. Weighed amounts of the crystals of the acid were added to the nutrient solution, which was then tubed and sterilized. Readings were made after one week's incubation at 25° C. All experiments which gave significant results were repeated at least once; some were repeated several times. When, however, a fungus made a good growth in the presence of 1 per cent tartaric acid, for example, it was not deemed necessary to repeat the work with 0.75 per cent concentration; or, if it failed to grow when the concentration of tartaric acid was 1 per cent, it was believed to be inadvisable to try 2 per cent acid more than once.

In view of the fact that tartaric acid does not kill the inoculum even when present in high concentrations, seven days was judged to be sufficient for the appearance of growth. Ordinarily, when no inhibiting substances are present in the nutrient solution, the inoculum shows growth within 24 hours. While it is probable that in some case an extension of the incubation period would have permitted growth, it is not the purpose of this paper to demonstrate whether or not these fungi can grow in the presence of a given concentration of tartaric acid, but rather to obtain a differential growth, subject to the limits of the experiment. The following table gives the results.

The same lack of uniformity characterizing the behavior of the different strains of a given species is apparent throughout table 6. Why should *F.*

TABLE 6.—*Growth in presence of various concentrations of tartaric acid (7 days at 25° C.)*

Organism	0.18%	0.37%	0.75%	1%	1.5%	2%	3%	4%	5%
natum 1	III–IV	I–II	I–I	0–I	0–0	0–0	0–0	0–0	0–0
natum 2–I	III–I	II–I–I	0–0–0	0	0	0	0	0	0
natum 2–II	IV–II	I–I	0–0	0	0	0	0	0	0
natum 2–III	III–II	III–II–0	I–0–0	0–0	0	0	0	0	0
natum 2–IV	III–II	II–I	0–0–0	0	0	0	0	0	0
natum 2–V	III–0	III–I–0	I–0–0	0–0	0	0	0	0	0
natum 2–VI	IV–III	III–I–I	I–0–0	0–0	0	0	0	0	0
natum 2–VII	V–III	IV–I	III–0–0	0–0	0	0	0	0	0
natum 2–VIII	IV–III	III–0–II	0–0	0	0	0	0	0	0
ıle	IV–III	I–I–II	0–0	0–0	0–0	0–0	0–0	0–0	0–0
›hilum	III	III–II	0–I–I	0–0	0	0	0	0	0
luctuum pusilum	II–0	II–0	II–0	II–0	II–0	II–0	0–0	0–0	0–0
luctuum volutum	0–I	0–0	0	0	0	0	0	0	0
um	0–II–I	0–0–0	0	0	0	0	0	0	0
›tium	V	V	V	V	IV–III	II–0	II–0	I–0–0	0–0
›iacum	V	V	V	IV	III–III	II–III	II–I	0–0	0
tis 1	V–II	V–IV	IV–0	IV–0	III–0	II–0	I–0	0–0	0
tis 2	V	V	IV–V	IV–V	III–III	II–I	I–0	0–0	0
enum	V	V	V	V	IV–III	II–III	II–I–I	0–0	0
ım I	V	V	IV	III–I–0	III–0–0	II–0–0	0–0	0	0
ım II	IV	III–III	0–II–II	0–I–0	0–0	0	0	0	0
ım brevius	V	V	III	III–II	II–I–0	0–I–0	0	0	0
›ceras I	V–V	IV–V	III–I–0	I–0–I	0–0	0	0	0	0
›ceras II	V	V	IV–I	II–0–I	0–0	0	0	0	0
um	IV	IV–II–0	III–0–0	II–0–0	0–0–0	0	0	0	0
'dosporum I	III	III	II–II	II–0–I	0–0–0	0	0	0	0
'dosporum II	III	III	II–II	II–II	I–0	I–I–0	0–0	0	0
'dosporum III	III	III–II	II–I	I–I	I–0	I–0	0–0	0	0
odinum	III–IV	II–II–0	0–0–0	0	0	0	0	0	0
um 1–I	III–IV	II–II–I	0–0–0	0	0	0	0	0	0
um 1–II	I–II–II	0–0–0	0	0	0	0	0	0	0
um 2	II–III	I–II	0	0	0	0	0	0	0
um 3	0–0–0	0–0	0	0	0	0	0	0	0
tatum	III–I	II–II–I	I–0–0	0–0	0	0	0	0	0
'inans	V	IV	IV	IV–III	II–0	I–0	0–0	0	0

TABLE 6.—*Continued*

Organism	0.18%	0.37%	0.75%	1%	1.5%	2%	3%	4%	5%
; 1	IV	IV	IV	III	II–0	I–II–0	0–0–0	0	0
; 2	V	V	V	V–IV	III–I	II–0	II–0–0	I–0	0–0
um 1–I	III	III–III	II–II	I–I	0–I	0	0	0	0
um 1–II	III	III–II	II–I	II–I	I–0	0	0	0	0
um 2	IV	IV–III	III–I	II	I–0	0–0	0	0	0
um 3–I	V–III	II–0–II	0–0	0	0	0	0	0	0
um 3–II	II–I–I	0–0–0	0	0	0	0	0	0	0
um 4–I	IV–II	III–0–II	0–0–0	0	0	0	0	0	0
um 4–II	III–III	III–0–0	0–0–0	0	0	0	0	0	0
um 5	III–I	I–0	0–0	0	0	0	0	0	0
um 6–I	V	V	V	V–V	IV–III	II–0–0	II–0–0	0	0
um 6–II	III–II	II–II	I–I	I–I	0	0	0	0	0
ellulare	I–0–0	0–0–0	0	0	0	0	0	0	0
m	III	III–III	0–0	0	0	0	0	0	0
sporum	IV	IV	III–0	II–0–0–I	0–0–0	0–0	0–0	0	0
me	II–II–I	0–0–0	0–0	0	0	0	0	0	0
ii 1–I	0–0	0–0	0	0	0	0	0	0	0
ii 1–II	0–IV–IV	0–II–II	0–0	0	0	0	0	0	0
ii 2–I	I–II–II	0–I–I	0	0	0	0	0	0	0
ii 2–II	II–II–II	0–0–0	0	0	0	0	0	0	0
ii 3	III–III	0–0	0	0	0	0	0	0	0
porum	V	V	IV–III	III–II	II–0–0	0–0	0	0	0
m	IV	III–II	II–I–I	0–0	0	0	0	0	0
m I	III–V	II–IV	I–0–I	0–0	0	0	0	0	0
m II	IV–V	IV–V	III–0–I	II–0–I	0–0	0	0	0	0
rum	0–V–V	0–0–I	0	0	0	0	0	0	0
enum	I–I–I	0–0–0	0	0	0	0	0	0	0
enum maius 1	III–IV–IV	0–I–I	0–0	0	0	0	0	0	0
enum maius 2–I	III–IV	II–I–0–0	0–0	0	0	0	0	0	0
enum maius 2–II	IV–IV	II–I–II–I	0–0	0	0	0	0	0	0
enum maius 2–III	III–IV–IV	0–0–I	0–0	0	0	0	0	0	0
enum maius 2–IV	IV–V	II–I–I	0–0	0	0	0	0	0	0
enum maius 2–V	III–III	III–II–I	0–0	0	0	0	0	0	0
enum maius 2–VII	IV	II–I	0–0	0	0	0	0	0	0
enum maius 2–VIII	III–IV–IV	II–I–I	0–0	0	0	0	0	0	0

TABLE 6.—*Continued*

Organism	0.18%	0.37%	0.75%	1%	1.5%	2%	3%	4%	5%
tigenum maius 3–I	II–II–II	I–0–0–0	0–0	0	0	0	0	0	0
tigenum maius 3–II	I–IV	0–0	0	0	0	0	0	0	0
tigenum maius 3–III	III–IV–III	0–0–0	0–0	0	0	0	0	0	0
osum	V	IV	III–0–II	II–0–I	I–0–0	0–0	0	0	0
inum 1	III–V	0–0	0	0	0	0	0	0	0
inum 2–I	III–IV	II–II	0–0	0	0	0	0	0	0
inum 2–II	IV–IV	IV–III	II–0–0	I–0–0	0	0	0	0	0
rum 1	0–I–II	0–0–I	0–0	0	0	0	0	0	0
rum 2	III	II–III–II	0–I–0	0	0	0	0	0	0
rum gibberalloides I	IV–V	III–IV	III–I	0–0	0	0	0	0	0
rum gibberalloides II	IV–V	III–V	III–I–III	0–0	0	0	0	0	0
rum pirinum I	II–II	II–0–0	0–0–0	0	0	0	0	0	0
rum pirinum II	II–II	II–0–0	0–0–0	0	0	0	0	0	0
oxysporum 1	V	V	V	V	IV–III	III–II	I–0	0	0
oxysporum 2	V	V	V	V–V	II–III	II–I	I–0	0	0
natum I	V	IV	III–II	II–I–I	0–0	0	0	0	0
natum II	V–V	IV–III	III–0	II–0–0	0–0	0	0	0	0
natum IV	V	V	III–I	I–I–0	0–I–0	0	0	0	0
natum V	V–V	IV–III	III–0–I	II–0–0	I–0–0	0–0	0	0	0
icum 1	IV	IV	III–II–II	I–0–I–0	0	0	0	0	0
icum 2	V	V	V	IV–III–I	III–II–0	II–0–0	II–0–0	I–0–0	0–0
tium I	0–0–0	0–0	0	0	0	0	0	0	0
tium II	0–II	0–0	0	0	0	0	0	0	0
	III	III–III–II	0–I–0	0	0	0	0	0	0
	III–III	II–II	II–I–I	I–0–I	0–0	0	0	0	0
ceras	V	V	IV	IV–III	III–III	II–0–III–0	I–0–I	0–0	0
ceras microsporum	V	V	V	V–V–III	IV–III–II	II–0–II	II–0–0	0–0	0
es	II–II	II–I–0	0–0	0	0	0	0	0	0
tum I	IV	IV	IV	III–IV	III–II	I–I	I–0	0–0	0
tum II	V	V	V	IV–IV	III–III	I–I	I–I	0–0	0
rsici 1	V	V	IV	V	III–II–IV	I–I–0	0–I	0	0
rsici 2	V	V	V	V–V	IV–III	III–0–II	I–0–0	0–0	0
	V	V	IV	III–IV	II–III	II–III	II–II	0–I	0
ceras	IV	III	III	III–I	II–0	II–I–0	0	0	0
1	III–IV	II–II–II	0–0	0	0	0	0	0	0

TABLE 6.—*Continued*

Organism	0.18%	0.37%	0.75%	1%	1.5%	2%	3%	4%	5%
i 2	V	V	V	V	IV	III	II–0	0–0	0
i 3–I	IV	II–II–II	0–II–I–0	I–0–0	0	0	0	0	0
i 3–II	IV	II	0–II	I	0	0	0	0	0
i minus	III–IV	II–II–II	0–0	0	0	0	0	0	0
i phaseoli 1–I	0–I–0	0–0	0	0	0	0	0	0	0
i phaseoli 1–II	0–V–0	0–II	0–I	0	0	0	0	0	0
i phaseoli 1–III	0–0–0	0–0	0	0	0	0	0	0	0
i phaseoli 2–I	0–0–0	0–0	0	0	0	0	0	0	0
i phaseoli 2–II	0–0–0	0	0	0	0	0	0	0	0
i pisi	III–V	II–V.	I–III–III	0–I–III	II	II	I–0	0	0
i viride	III	II–III–III	0–I–II	0–0	0	0	0	0	0
iforme 1	V	V	V	V	IV	IV	III–I	II–0–I	II–0–0
iforme 2	IV	IV	IV	IV	IV	III–I	II–0	I–0–0	I–0–0
iforme 3	0–III	0–0	0	0	0	0	0	0	0
iforme 4	V	V	V	V–IV	IV–III	III–0–II	I–0–0	0–0	0
iforme 5	V	V	V	V	IV–IV	IV–IV–0	III–0–II	I–0–0–II	I–0–0
iforme erumpens I	V	V	V	IV	IV–III	III–II–0	II–0–0	0–0	0
iforme erumpens II	V	V	V	V	IV	IV–I	II–I–0	I–0–0	0–0
iforme minus	V	IV	IV	IV	IV	IV–0–III	III–I–0	II–I–0	0–0–0
iforme subglutinans	V	V	IV	IV	III–III	II–II	I–I–I	0–0	0
ras	V	IV	IV	IV–IV	III–II	II–0	0–0	0	0
m 1	V	V	V	IV	IV–0	III–I–0	II–0–0	0–0	0
m 2	V	V	IV	III	II–0	II–I–0	0–0	0–0–0	0
ceras	IV	IV	IV	III–III	II–II	II–I–I	I–0–I	0–0	0
ceras trisceptatum	V	V	V	IV	IV–IV	II–0–III	II–I–I–0	0–I–0	0
lum 1	IV	IV	IV	IV–0	III–0	II–I–0	0–0	0	0
lum 2	V–V	III–0–II	0–0–0	0	0	0	0	0	0
orum 1	V	V	V	IV–IV	II–I	II–0	I–0–0	0–0	0
orum 2	V	V	V	IV–IV	III–0	II–I	I–0	0–0	0
orum nicotianae	V	V	V	IV–0	III–III	II–II	I–I	0–0	0
licola	III–III	II–II	I–I	0–0	0	0	0	0	0
um	V–V	III–IV	II–0–I	0–0	0	0	0	0	0
I	III–II–III	0–0–I	0	0	0	0	0	0	0
II	II–IV	0–III–III	0–0	0	0	0	0	0	0
iorphum	III–IV	II–I–II	0–0	0	0	0	0	0	0

TABLE 6.— (*Continued*)

Organism	0.18%	0.37%	0.75%	1%	1.5%	2%	3%	4%	5%
sillum	IV–IV	III–IV–IV	0–I–0	0	0	0	0	0	0
dicicola	IV–V–II	II–II–II	0–0	0–0	0	0	0	0	0
izochromatistes	V	V	V	V–V	IV–IV	III–0–III	III–0–I	0–0–0	0
izochromatistes- *roselerotium*	V–V	IV–V	IV–III	III–III	II–0–II	II–0–I	0–0–0	0	0
mbucinum 1	III–IV	II–II–III	0–I–0	0	0	0	0	0	0
mbucinum 2	IV	IV–III	III–II	II–I–II	I–0	0–0	0	0	0
rpi	III–III	II–II–III	0–0	0	0	0	0	0	0
erostromaton	V	V	V	IV–IV	IV–I	II–I–0	II–0–0	0–0–0	0
nitectum 1–I	IV–IV–IV	IV–IV–IV	III–II–I	II–I–I	I–I–0	0–0	0	0	0
nitectum 1–II	V–V–V	V–IV–II	IV–I–II	II–0–I	I–0	0	0	0	0
nitectum 1–III	IV–V–V	IV–III–IV	III–II–II	II–II	I–I	0–0	0	0	0
nitectum 1–IV	IV–IV–V	III–IV–III	III–I–0	II–0–0	0–0	0	0	0	0
nitectum 1–V	IV–IV–III	III–0–II	III–0–I	II–0–I	0–0–0	0	0	0	0
nitectum 1–VI	V–III	IV–II–II	III–0–0	II–0–0	II–0–0	0	0	0	0
nitectum 1–VII	III–IV–IV	II–0–I	I–0–I	0–0–I	0–0	0	0	0	0
nitectum 1–VIII	V–V	V–V	V–V	V–II–V	IV–II–0–III	II–II–0–I	II–0–I	0	0
nitectum 1–IX	V–V–III	0–II–III	I–I	0–0	0–0	0–0	0–0	0	0
nitectum 1–X	V–V–V	V–IV	II–II–III	0–0–I	0–0	0–0	0	0	0
nitectum 1–XI	V–V–V	V–III	I–II–II	0–0–0	0	0	0	0	0
nitectum 1–XII	V–IV–III	IV–III	I–I–II	0–I	0	0	0	0	0
nitectum 1–XIII	V–IV	III–III	0–II	I–I–0	0–I–0	0	0	0	0
nitectum 1–XIV	III–III–II	0–0–I	0–0	0–0	0	0	0	0	0
nitectum 1–XV	III–0–III	0–0–I	0–0–I	0	0	0	0	0	0
nitectum 1–XVI	IV–III–III	0–II	I–I	0–0	0–I	0	0	0	0
nitectum 1–XVII	V–V–IV	IV–IV	III–III–III	II–0	I–0	0–0	0–0	0–0	0
nitectum 1–XVIII	IV–V	III–III	II–III	0–0	0–0	0	0	0	0
nitectum 2	IV	IV	III–III	II–I–II	I–I–0	0–0	0	0	0
mi 1	III–IV	II–II–II	0–0	0	0	0	0	0	0
mi 2–I	V	V	IV	III–IV	II–III	II–II	0	0	0
mi 2–II	V–V	V–I–III	IV–0–II	III–0–I	II–0–0	II–0–0	0–0	0	0
mi 3–I	V	III	III–I	II–I–0	II–0–0	0–0	0	0	0
mi 3–II	V–V	IV–III–II	0–0–0	0	0	0	0	0	0
mi 3–III	II–V–V	I–IV–0	0–II–0	0	0	0	0	0	0
mi 4–I	I–III–I	I–0–0	0–0	0	0	0	0	0	0

TABLE 6.—(*Continued*)

Organism	0.18%	0.37%	0.75%	1%	1.5%	2%	3%	4%	5%
ni 4–II	IV–IV	III–II–I	II–I–0	II–0–0	0	0	0	0	0
ni minus 1–I	II–V	II–IV–II	0–0–0	0	0	0	0	0	0
ni minus 1–II	IV–IV	III–II–II	0–0–0	0	0	0	0	0	0
ni minus 2–I	IV–III	II–II–I	I–0–0	0–0	0	0	0	0	0
ni minus 2–II	II–II–II	0–0–I	0	0	0	0	0	0	0
ni suffuscum	III–IV	II–0–II	0–0	0	0	0	0	0	0
aceae 1	V	V	IV	IV–III	III–III	II–II	0	0	0
aceae 2	V	V	V	V	IV–II	III–I	II–I	I–0	0–0
atum 1–I	III–IV–III	0–II–II	0	0	0	0	0	0	0
atum 1–II	0–0–0	0–0–0	0	0	0	0	0	0	0
atum 2	III–III	II–II	I–I–I	0–0	0	0	0	0	0
atum 3–I	IV–III–IV	II–II–II	0–0–0	0	0	0	0	0	0
atum 3–II	I–0–V	0–0–III	0–0	0–0	0	0	0	0	0
hureum 1	III–I–IV	I–0–0	0–0	0	0	0	0	0	0
hureum 2	IV–IV	II–I–II	0–0	0	0	0	0	0	0
hureum 3	IV–V	III–IV–IV	0–I–I	I–0	0–0	0	0	0	0
brome	IV–IV	III–II	II–II	0–0	0	0	0	0	0
heiphilum	V	V	V	V–V	II–0	II–0	II–0–0	I–0	0–0
hothecioides 1	II–IV–II	0–I–0	0	0	0	0	0	0	0
hothecioides 2–I	V–II	II–0	0–0	0	0	0	0	0	0
hothecioides 2–II	V	III–III	I–0	0–0	0	0	0	0	0
hothecioides 2–III	II–I–0	0–0–0	0–0	0	0	0	0	0	0
hothecioides 3–I	V	III–II	III–0–II	II–0–0	0–0	0	0	0	0
hothecioides 3–II	I–II	I–0	0–0	0	0	0	0	0	0
hothecioides 4–I	0–I	0	0	0	0	0	0	0	0
hothecioides 4–II	III–IV–IV	0–IV–III	0–0–II	0–0	0–0	0	0	0	0
hothecioides 5	V	V	V	III–II	II–0	II–0	I–0	0–0	0
hothecioides 6	V	V	V–V	IV–V	II–II	0–I	0	0	0
hothecioides 7–I	II–IV–II	0–0–0	0	0	0	0	0	0	0
hothecioides 7–II	I–V–III	0–0–0	0	0	0	0	0	0	0
inctum 1–II	III–IV–IV	I–II–III	0–0–II	0	0	0	0	0	0
inctum 1–III	II–IV	II–III–III	0–0–I	0	0	0	0	0	0
inctum 1–IV	IV	III	II–I	0–0	0	0	0	0	0
inctum 1–V	IV–V	III–IV	II–0–I	0–0	0	0	0	0	0
inctum 1–VI	III–V	II–V	I–0–V	0–0–0	0	0	0	0	0

TABLE 6.— (*Continued*)

Organism	0.18%	0.37%	0.75%	1%	1.5%	2%	3%	4%	5%
cinctum 1–VII	II–0–III	0–0–I–II	0–0	0	0	0	0	0	0
cinctum 1–VIII	III–III	II–II	0–0	0	0	0	0	0	0
cinctum 1–IX	III–IV	I–II–III	0–0–I	0	0	0	0	0	0
cinctum 2	III–III–III	I–I–I	0–0–0	0	0	0	0	0	0
cinatum	IV	IV	IV–III	III–III	II–I–II	0–I	0–0	0	0
icearum I	II–V–III	0–0–I	I–0	0–0	0	0	0	0	0
icearum II	0–I–0	0–0–0	0	0	0	0	0	0	0
infectum	V	V	V	IV–III	II–I	II–I–I	I–0–0	I–0–0	0–0
ide 1	IV–IV	II–0–II	0	0	0	0	0	0	0
ide 2	V	V	V	V	IV–IV	III–0	II–0	I–0	0–0
icola	IV–IV	III–III–III	I–I–II	I–0	0	0	0	0	0
atum 1	III	III	II	II–II–II	0–I–0	0	0	0	0
atum 2	V	V	V	V–II	III–0	II–0	I–I	0–0	0
atum 3	V	V	V	V	IV–III	II–0	I–I–0	0–0	0

coeruleum 3 fail to grow in presence of 0.18 per cent tartaric acid, while *F. coeruleum* 1–I, 1–II and 2 grow fairly well? Why should *F. culmorum* 6–I make an excellent growth in presence of 1.5 per cent of the acid, while *F. culmorum* 3–II fails to make any growth, even when the concentration of tartaric acid is no more than 0.37 per cent? Of the five strains of *F. eumartii,* no. 1–I is the only one unable to grow in the presence of 0.18 per cent tartaric acid. *F. martii* 1–I is totally inhibited by 0.75 per cent of the acid, while *F. martii* 2 is not inhibited, even by 2 per cent concentration of the same acid. Of all the strains of *F. moniliforme,* no. 3 is the only one which is inhibited by a concentration of only 0.37 per cent of tartaric acid, while the others withstand as much as 2 per cent or more. *F. semitectum* 1–VIII tolerates more acid than any of the other strains. *F. solani* 2–I withstands 2 per cent of the acid, while 3–II and 4–I show no sign of growth when the amount of acid present is more than 0.37 per cent. *F. striatum* 1–I grows in the presence of 0.18 and even 0.37 per cent of the acid, while no. 1–II does not. *F. trichothecioides* 4–I can hardly tolerate 0.18 per cent of the acid, while no. 6 grows even when the concentration is increased to 1.5 per cent. *F. viride* 2 can tolerate as much as 1.5 and 2 per cent of tartaric acid, while the upper limit of acid tolerance for no. 1 is not more than 0.37 per cent.

On the other hand, there are numerous instances where the different strains of the same species exhibit a remarkably uniform set of reactions. All strains of *F. acuminatum* tolerate only small amounts of acid; the same applies to *F. fructigenum* and its varieties. With only one exception all the strains of *F. moniliforme* can grow in the presence of high concentrations of tartaric acid. Both strains of *F. batatatis,* of *F. camptoceras,* the three strains of *F. chlamydosporum, F. cubense* 1 and 2, *F. herbarum gibberalloides* I and II, *F. hyperoxysporum* 1 and 2, *F. lutulatum* I and II, *F. lycopersici* 1 and 2, and many others agree very closely in their reactions toward tartaric acid.

Because of the inconstancy of results noted in many cases, this type of fluctuating variation was tested further in an extensive series of experiments. It was deemed advisable also to make certain that such conflicting results as are listed in the foregoing table were not caused by any error in technique but were attributable to the nature of the living protoplasm. All organisms showing strikingly conflicting reactions were studied for two generations to determine whether a more comprehensive range of variations could be established. If, for example, a given inoculum failed to make any growth in the presence of 2 per cent tartaric acid, it was subsequently transferred to a tube of nutrient solution void of any inhibiting substance. After a good growth was obtained, it was transferred to agar plates and inoculum discs were cut from the resulting colony and transferred to a series of tubes

of nutrient solution containing 1.5, 2, and 3 per cent of tartaric acid. The results are given below. Arrows indicate the course of procedure from beginning to end. For instance, an inoculum disc cut from a colony of *F. asclerotium* and transferred to 2 c.c. of nutrient solution containing 2 per cent tartaric acid fails to make any growth. When, after 7 days, it is transferred to a nutrient solution containing no tartaric acid, it begins to grow vigorously, showing that it was not killed by the acid but merely inhibited. A transfer is made from this growth to a plate of agar and from the resulting colony inoculum discs are cut and transferred to 0.75, 1, 1.5, and 2 per cent tartaric acid solutions; all of them grow, as indicated by the numerals V, IV, III and II. The following pages illustrate such reactions in condensed form.

The foregoing facts need no further explanation. The organisms used are numerous enough to exclude the possibility of accidental mixtures or contaminations. Some of the experiments have been repeated a number of times from every angle, to eliminate, as much as possible, any environmental factor that may have been introduced into the work. It seems, therefore, that the cause for the extreme fluctuations exhibited by these organisms is to be found not in the external environmental factors but within the mechanism of the protoplasm, itself.

Reproduction in the Presence of Various Concentrations of Tartaric Acid

Microscopic examination of tartaric-acid cultures was made seven days after the inoculum was transferred to the different solutions. An attempt was made to secure representative portions of the mycelium in order not to miss any of the spore types, because sometimes it so happens that not all of the spores can be found together in any one region. Furthermore, when reproduction is very sparse, it becomes necessary to examine quite a bit of the mycelial mass in order to ascertain the abundance of the different spore forms.

The writer does not accept spore production as a dependable specific character; the growth factor, being more stable, should be of greater service in physiological taxonomy. However, the effect of tartaric acid upon reproduction in Fusaria presents some interesting biological phenomenon as can be seen in the following tables.

The foregoing table suggests the following generalization: Of the three types of asexual sporulation in the genus Fusarium, chlamydospore production is the most easily inhibited by tartaric acid, and production of microconidia is inhibited the least. It can be speculatively stated that the degree of sensitivity to unfavorable external environments is indicative of the comparative age of these spores in the evolutionary life of Fusarium species.

F. asclerotium → 2 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.75 per cent tartaric acid = V
- → 1 " " " = IV
- → 1.5 " " " = III
- → 2 " " " = II

F. aquaeductuum pusilum → 0.75 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.18 per cent tartaric acid = 0
- → 0.35 " " " = 0
- → 0.75 " " " = 0
- → 1 " " " = 0

F. batatatis 1 → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.75 per cent tartaric acid = 0
- → 1 " " " = 0
- → 1.5 " " " = 0
- → 2 " " " = 0

F. bullatum I → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.75 per cent tartaric acid = II
- → 1 " " " = 0
- → 1.5 " " " = 0
- → 2 " " " = 0

F. chenopodinum → 0.37 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.18 per cent tartaric acid = 0
- → 0.35 " " " = 0
- → 0.75 " " " = 0
- → 1 " " " = 0

F. conglutinans → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.75 per cent tartaric acid = IV
- → 1 " " " = III
- → 1.5 " " " = III

F. cubense 1 → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.75 per cent tartaric acid = V
- → 1 " " " = III
- → 1.5 " " " = III
- → 2 " " " = II

F. caudatum → 0.75 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.18 per cent tartaric acid = IV
- → 0.35 " " " = 0
- → 0.75 " " " = 0
- → 1 " " " = 0

F. camptoceras I → 0.75 per cent tartaric acid = III → agar
- → 0.18 per cent tartaric acid = V
- → 0.35 " " " = V
- → 0.75 " " " = 0

F. diversisporum I → 0.75 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.18 per cent tartaric acid = V
- → 0.35 " " " " = II
- → 0.75 " " " " = 0
- → 1 " " " " = 0

F. euoxysporum → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.75 per cent tartaric acid = III
- → 1 " " " " = I
- → 1.5 " " " " = 0
- → 2 " " " " = 0

F. lycopersici 2 → 2 per cent tartaric acid = 0 → nutrient solution → agar
- → 1 per cent tartaric acid = V
- → 1.5 " " " " = IV
- → 2 " " " " = II
- → 3 " " " " = 0

F. lycopersici microsporum → 2 per cent tartaric acid = 0 → nutrient solution → agar
- → 1 per cent tartaric acid = III
- → 1.5 " " " " = II
- → 2 " " " " = 0
- → 3 " " " " = 0

F. loncheceras microsporum → 3 per cent tartaric acid = 0 → nutrient solution → agar
- → 2 per cent tartaric acid = 0
- → 3 " " " " = 0
- → 4 " " " " = 0

F. loncheceras

2 per cent tartaric acid = 0 → nutrient solution → agar
- → 1 per cent tartaric acid = V
- → 1.5 " " " " = III
- → 2 " " " " = 0
- → 3 " " " " = 0

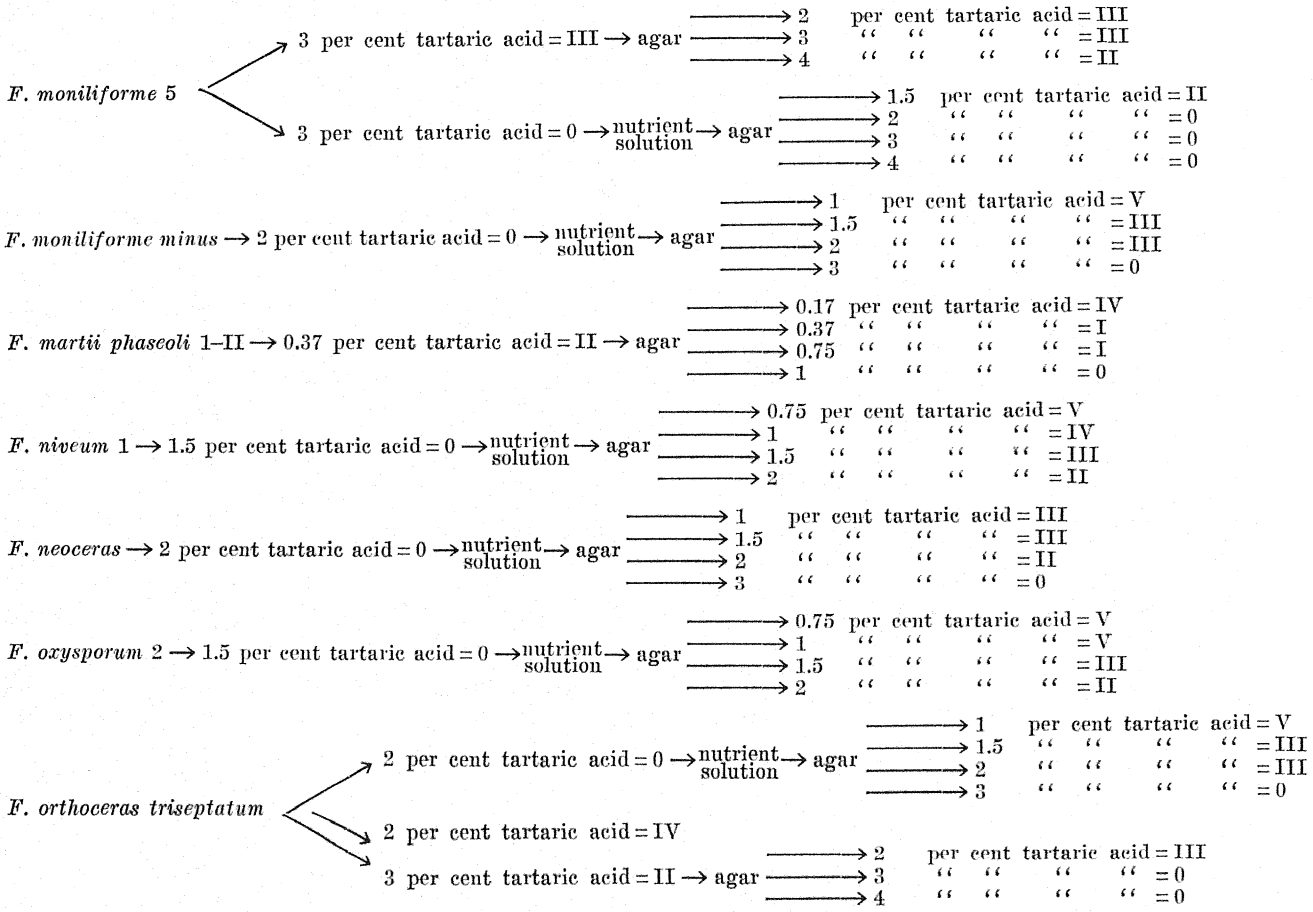
2 per cent tartaric acid = II → agar
- → 1.5 per cent tartaric acid = III
- → 2 " " " " = III
- → 3 " " " " = 0

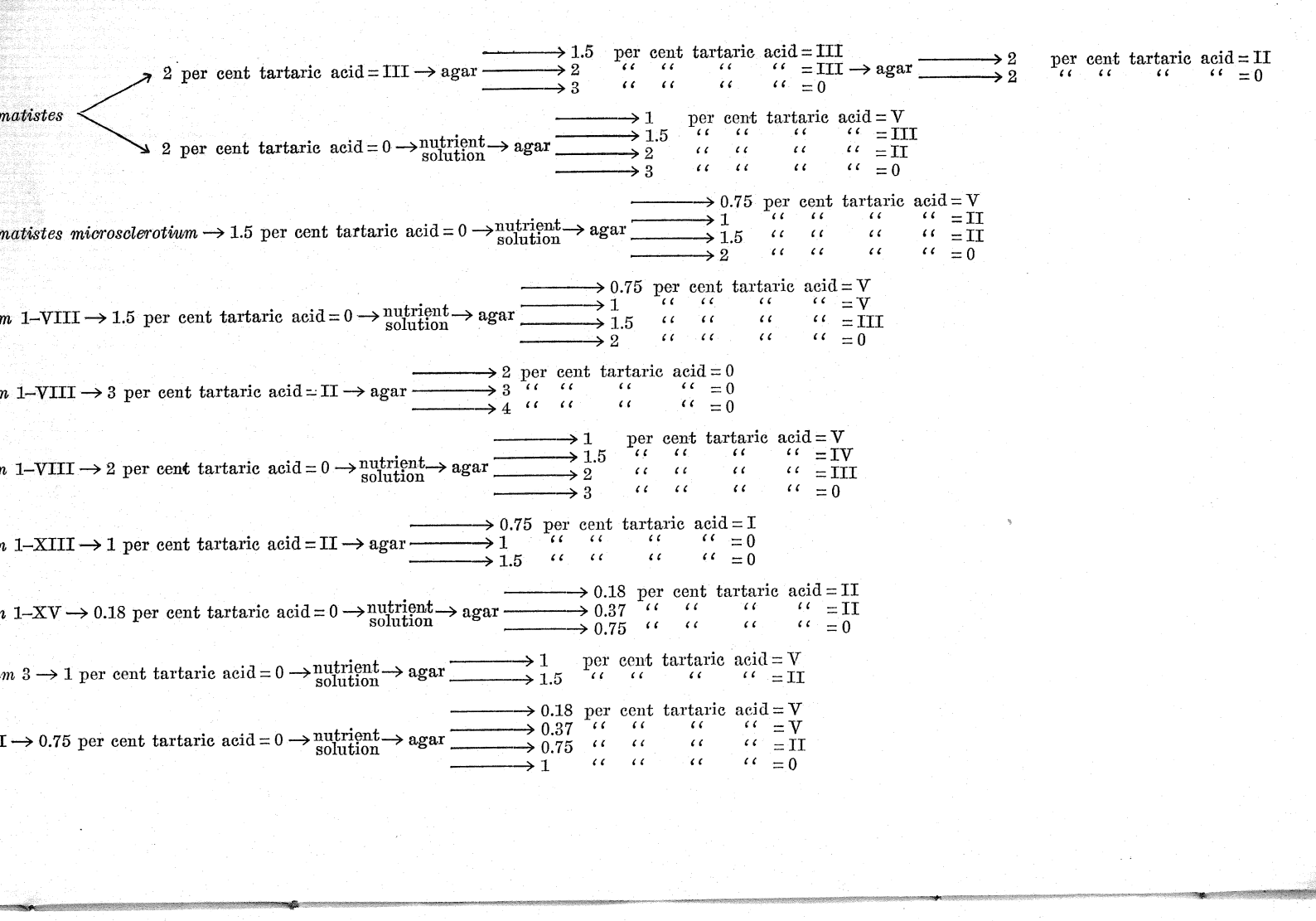
F. martii phaseoli 1–II → 0.18 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.18 per cent tartaric acid = V
- → 0.37 " " " " = II
- → 0.75 " " " " = 0

F. moniliforme 4

2 per cent tartaric acid = III → agar
- → 1.5 per cent tartaric acid = IV
- → 2 " " " " = III
- → 3 " " " " = 0

2 per cent tartaric acid = 0 → nutrient solution → agar
- → 1 per cent tartaric acid = V
- → 1.5 " " " " = IV
- → 2 " " " " = 0
- → 3 " " " " = 0


F. moniliforme 5
3 per cent tartaric acid = III → agar
→ 2 per cent tartaric acid = III
→ 3 " " = III
→ 4 " " = II
3 per cent tartaric acid = 0 → nutrient solution → agar
→ 1.5 per cent tartaric acid = II
→ 2 " " = 0
→ 3 " " = 0
→ 4 " " = 0
F. moniliforme minus → 2 per cent tartaric acid = 0 → nutrient solution → agar
→ 1 per cent tartaric acid = V
→ 1.5 " " = III
→ 2 " " = III
→ 3 " " = 0
F. martii phaseoli 1–II → 0.37 per cent tartaric acid = II → agar
→ 0.17 per cent tartaric acid = IV
→ 0.37 " " = I
→ 0.75 " " = I
→ 1 " " = 0
F. niveum 1 → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
→ 0.75 per cent tartaric acid = V
→ 1 " " = IV
→ 1.5 " " = III
→ 2 " " = II
F. neoceras → 2 per cent tartaric acid = 0 → nutrient solution → agar
→ 1 per cent tartaric acid = III
→ 1.5 " " = III
→ 2 " " = II
→ 3 " " = 0
F. oxysporum 2 → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
→ 0.75 per cent tartaric acid = V
→ 1 " " = V
→ 1.5 " " = III
→ 2 " " = II
F. orthoceras triseptatum
2 per cent tartaric acid = 0 → nutrient solution → agar
→ 1 per cent tartaric acid = V
→ 1.5 " " = III
→ 2 " " = III
→ 3 " " = 0
2 per cent tartaric acid = IV
3 per cent tartaric acid = II → agar
→ 2 per cent tartaric acid = III
→ 3 " " = 0
→ 4 " " = 0


matistes
2 per cent tartaric acid = III → agar
1.5 per cent tartaric acid = III
2 " " " " = III → agar
3 " " " " = 0
2 per cent tartaric acid = II
2 " " " " = 0
2 per cent tartaric acid = 0 → nutrient solution → agar
1 per cent tartaric acid = V
1.5 " " " " = III
2 " " " " = II
3 " " " " = 0
matistes microsclerotium → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
0.75 per cent tartaric acid = V
1 " " " " = II
1.5 " " " " = II
2 " " " " = 0
m 1–VIII → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
0.75 per cent tartaric acid = V
1 " " " " = V
1.5 " " " " = III
2 " " " " = 0
n 1–VIII → 3 per cent tartaric acid = II → agar
2 per cent tartaric acid = 0
3 " " " " = 0
4 " " " " = 0
n 1–VIII → 2 per cent tartaric acid = 0 → nutrient solution → agar
1 per cent tartaric acid = V
1.5 " " " " = IV
2 " " " " = III
3 " " " " = 0
n 1–XIII → 1 per cent tartaric acid = II → agar
0.75 per cent tartaric acid = I
1 " " " " = 0
1.5 " " " " = 0
n 1–XV → 0.18 per cent tartaric acid = 0 → nutrient solution → agar
0.18 per cent tartaric acid = II
0.37 " " " " = II
0.75 " " " " = 0
m 3 → 1 per cent tartaric acid = 0 → nutrient solution → agar
1 per cent tartaric acid = V
1.5 " " " " = II
I → 0.75 per cent tartaric acid = 0 → nutrient solution → agar
0.18 per cent tartaric acid = V
0.37 " " " " = V
0.75 " " " " = II
1 " " " " = 0

F. solani 3–III → 0.75 per cent tartaric acid = II → agar
- → 0.37 per cent tartaric acid = 0
- → 0.75 " " " " = 0
- → 1 " " " " = 0

F. solani 3–III → 0.37 per cent trataric acid = 0 → nutrient solution → agar
- → 0.18 per cent tartaric acid = V
- → 0.37 " " " " = V
- → 0.75 " " " " = 0

F. solani 4–I
- → 0.18 per cent tartaric acid = II → agar
  - → 0.18 per cent tartaric acid = 0
  - → 0.37 " " " " = II
  - → 0.75 " " " " = 0
- → 0.18 per cent tartaric acid = 0 → nutrient solution → agar
  - → 0.18 per cent tartaric acid = I
  - → 0.18 " " " " = V

F. tricinctum 1–VII → 0.18 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.18 per cent tartaric acid = IV
- → 0.37 " " " " = II
- → 0.75 " " " " = 0

F. viride 2 → 2 per cent tartaric acid = 0 → nutrient solution → agar
- → 1 per cent tartaric acid = V
- → 1.5 " " " " = V
- → 2 " " " " = III
- → 3 " " " " = I

F. zonatum 2 → 1.5 per cent tartaric acid = 0 → nutrient solution → agar
- → 0.75 per cent tartaric acid = III
- → 1 " " " " = III
- → 1.5 " " " " = II
- → 2 " " " " = 0

F. zonatum 3 → 2 per cent tartaric acid = 0 → nutrient solution → agar
- → 1 per cent tartaric acid = III
- → 1.5 " " " " = III
- → 2 " " " " = 0
- → 3 " " " " = 0

TABLE 7.—*Reproduction in the presence of various concentrations of tartaric acid. 0.18%–1.5%*[10]

anism	.18%			.37%			.75%			1%			1.5%		
	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.
2–III	II	III	0	I	II	0	0	0	0	0	0	0	0	0	0
2–IV	0	I	0	0	0	0	0	0	0	0	0	0	0	0	0
2–VII	0	I	0	0	0	0	0	0	0	0	0	0	0	0	0
2–VIII	0	III	0	0	III	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0
n pusilum	III	0	0	III	0	0	0–III	0	0	0–III	0	0	0–II	0	0
n volutum II	III	0	0	III	0	0	III	0	0	II	0	0	II	0	0
	III	0	0	III	0	0	III	0	0	III	0	0	III	0	0
	IV	I	III	IV	I	III	IV	0	0	IV	0	0	III	0	0
	IV	0	0	IV	0	0	IV	0	0	0	0	0	0	0	0
	IV	II	0	IV	II	0	IV	II	0	III	0	0	III	0	0
	V	II	III	V	0	III	V	0	III	IV	0	0	IV	0	0
	I	IV	0	0	III	0	0	0	0	0	0	0	0	0	0
vius	III	III	0	III	III	0	0	0	0	0	0	0	0	0	0
I	II	IV	0	0	0	0	0	0	0	0	0	0	0	0	0
II	I	III	0	0	0	0	0	0	0	0	0	0	0	0	0
	I	I	0	I	I	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	I	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	III	0	0	II	0	0	II	0	0	0	0	0
	IV	III	III	IV	II	III	IV	II	0	IV	II	0	0	0	0
	III	0	0	III	0	0	II	0	0	0	0	0	0	0	0
	0	IV	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	II	II	III	II	II	III	I	I	III	0	0	0	0	0
I	0–II	IV	0	0–II	IV	0	0	0	0	0	0	0	0	0	0
II	I–IV	0–II	0	0–IV	0–II	0	0–IV	0–II	0	0	0	0	0	0	0
	0–III	0–0	0–0	0	0	0	0	0	0	0	0	0	0	0	0
	III	II	0	II	II	0	0	0	0	0	0	0	0	0	0
	0–0	0–IV	0–0	0	0	0	0	0	0	0	0	0	0	0	0
2	IV	0	0	IV	0	0	IV	0	0	III	0	0	0	0	0
naius 1	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
naius 2–V	0	V	0	0–0	I–III	0–0	0	0	0	0	0	0	0	0	0

e organisms which formed spores in the presence of tartaric acid have been included in this and the next table.

TABLE 7.—(*Continued*)

anism	.18%			.37%			.75%			1%			1.5%		
	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Ch
maius 3–III	0	II	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	II	0	IV	II	0	IV	0	0	IV	0	0	III	0	0
	III	II	II	III	0	0	III	0	0	0	0	0	0	0	0
–II	I	IV	0	I	III	0	0	0	0	0	0	0	0	0	0
bberalloides I	II	0	0	I	0	0	I	0	0	0	0	0	0	0	0
bberalloides II	III	II	0	III	II	0	0	0	0	0	0	0	0	0	0
rum 1	V	IV	IV	V	IV	IV	IV	IV	0	IV	IV	0	III	0	0
rum 2	IV	III	0	IV	III	0	IV	II	0	III	0	0	0	0	0
I	II	IV	0	0	0	0	0	0	0	0	0	0	0	0	0
II	II	IV	0	II	III	0	II	III	0	0	0	0	0	0	0
IV	II	IV	0	II	IV	0	II	II	0	0	0	0	0	0	0
V	II	IV	0	II	IV	0	0	0	0	0	0	0	0	0	0
	II	I	III	0	0	0	0	0	0	0	0	0	0	0	0
	V	0	II	V	0	II	V	0	0	III	0	0	II	0	0
	III	0	0	III	0	0	III	0	0	III	0	0	III	0	0
	IV	IV	0	IV	IV	0	IV	IV	0	IV	III	0	III	0	0
I	III	V	0	III	V	0	III	IV	0	III	III	0	III	II	0
2	III	II	0	III	I	0	III	0	0	III	0	0	II	0	0
	II	0	0	II	0	0	II	0	0	0	0	0	0	0	0
	0	II	0	0	0	0	0	0	0	0	0	0	0	0	0
	III	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	0	I	IV	0	I	III	0	0	III	0	0	II	0	0
	0–III	0	0	0	0	0	0	0	0	0	0	0	0	0	0
eoli 1–II	0–IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	V	III	0	V	III	0	III	0	0	0	0	0	0	0	0
1	IV	0	0	IV	0	0	IV	0	0	IV	0	0	IV	0	0
2	IV	0	0	IV	0	0	IV	0	0	IV	0	0	III	0	0
5	IV	0	0	IV	0	0	IV	0	0	IV	0	0	IV	0	0
erumpens I	III	0	0	III	0	0	III	0	0	III	0	0	II	0	0
erumpens II	IV	0	0	IV	0	0	IV	0	0	IV	0	0	IV	0	0
minus	IV	I	0	IV	0	0	IV	0	0	IV	0	0	II	0	0
subglutinans	III	0	0	III	0	0	III	0	0	III	0	0	II	0	0
	III	0	0	III	0	0	III	0	0	III	0	0	III	0	0

TABLE 7.—(*Continued*)

Organism	.18%			.37%			.75%			1%			1.5%		
	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	C
	III	III	II	III	II	II	III	II	0	II	0	0	0	0	0
	II	0	0	II	0	0	0	0	0	0	0	0	0	0	0
triseptatum	III	0	III	III	0	III	III	0	III	III	0	0	II	0	0
1	V	I	0	IV	I	0	IV	0	0	IV	0	0	IV	0	0
2	III	IV	0	I	II	0	0	0	0	0	0	0	0	0	0
1	II	I	III	II	0	II	II	0	0	II	0	0	0	0	0
2	V	0	II	V	0	0	IV	0	0	IV	0	0	IV	0	0
nicotianae	IV	0	II	IV	0	II	IV	0	0	IV	0	0	0	0	0
	II	II	0	II	II	0	0	0	0	0	0	0	0	0	0
natistes	V	II	III	V	II	I	IV	0	0	III	0	0	III	0	0
natistes-erotium	IV	0	0	III	0	0	III	0	0	0	0	0	0	0	0
m 2	III	0	0	III	0	0	0	0	0	0	0	0	0	0	0
naton	III	II	II	III	II	0	III	0	0	II	0	0	II	0	0
1–I	IV	IV	I–0	II	II	0	0	0	0	0	0	0	0	0	0
1–II	IV	III	0	III	III	0	II	II	0	0	0	0	0	0	0
1–III	IV	IV	0	III	III	0	II	II	0	0	0	0	0	0	0
1–IV	I	I	0	I	I	0	I	0	0	0	0	0	0	0	0
1–VI	III	II	0	II	II	0	II	I	0	0	0	0	0	0	0
1–VIII	V	III	II	IV	III	II	IV	II	II	III	0	0	III	0	0
1–X	III	IV	0	III	IV	0	0	0	0	0	0	0	0	0	0
1–XI	IV	III	0	0–IV	0–III	0	0	0	0	0	0	0	0	0	0
1–XII	II–IV	II–IV	0–0	II–III	II–II	0–0	0	0	0	0	0	0	0	0	0
1–XIII	II–IV	II–IV	0–0	II–III	II–II	0–0	0	0	0	0	0	0	0	0	0
1–XVII	II	III	0	I	II	0	0	0	0	0	0	0	0	0	0
2	II	IV	0	II	III	0	0	0	0	0	0	0	0	0	0
	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	V	0	0	V	0	0	IV	0	0	II	0	0	0	0	0
[	V	III	II	V	III	II	III	I	I	II	0	0	0	0	0
	IV	IV	0	III	0	0	0	0	0	0	0	0	0	0	0
[	IV	II	0	IV	0	0	0	0	0	0	0	0	0	0	0
I	III	II	III	III	0	0	0	0	0	0	0	0	0	0	0
	0–II	0–0	0–0	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 7.—(*Continued*)

rganism	.18%			.37%			.75%			1%			1.5%		
	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.	Mic.	Mac.	
[	III	V	0	III	0	0	0	0	0	0	0	0	0	0	0
us 1–I	0–V	0–0	0–0	0–IV	0–0	0–0	0	0	0	0	0	0	0	0	0
us 1–II	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0
us 2–I	IV	0	0	0	0	0	0	0	0	0	0	0	0	0	0
us 2–II	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	IV	III	III	IV	III	III	IV	III	0	III	III	0	0	0	0
2	IV	III	III	IV	III	III	IV	III	III	IV	III	0	II	0	0
–I	III	II	0	0	0	0	0	0	0	0	0	0	0	0	0
–II	0–III	0–III	0–0	0	0	0	0	0	0	0	0	0	0	0	0
ı 2	I	III	0	0	0	0	0	0	0	0	0	0	0	0	0
ı 3	0	III	0	0	I	0	0	0	0	0	0	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0
ıum	V	0	0	V	0	0	V	0	0	V	0	0	0	0	0
oides 1–I	I	0	0	0	0	0	0	0	0	0	0	0	0	0	0
oides 2–II	0	III	0	0	0	0	0	0	0	0	0	0	0	0	0
oides 3–I	I	III	0	0	0	0	0	0	0	0	0	0	0	0	0
oides 4–II	0–0	0–V	0–0	0–0	0–V	0–0	0	0	0	0	0	0	0	0	0
oides 5	V	0	0	V	0	0	IV	0	0	III	0	0	0	0	0
oides 6	IV	II	0	IV	II	0	IV	II	0	IV	II	0	II	0	0
oides 7–II	0–II	0–I	0–0	0	0	0	0	0	0	0	0	0	0	0	0
1–III	III	III	I	III	I	0	0	0	0	0	0	0	0	0	0
1–IV	III	0	0	III	0	0	0	0	0	0	0	0	0	0	0
1–V	III	II	0	III	0	0	0	0	0	0	0	0	0	0	0
1–VI	III–V	0–II	0–0	0–V	0–II	0–0	0–V	0–II	0–0	0	0	0	0	0	0
1–VII	0–IV	0–II	0–0	0	0	0	0	0	0	0	0	0	0	0	0
1–IX	III	II	0	0	0	0	0	0	0	0	0	0	0	0	0
2	II	II	0	0	0	0	0	0	0	0	0	0	0	0	0
	IV	III	0	IV	III	0	III	II	0	III	0	0	0	0	0
ı I	0–II	0–V	0–0	0	0	0	0	0	0	0	0	0	0	0	0
m	II	IV	IV	II	IV	0	II	IV	0	II	IV	0	0	0	0
	II	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	V	0	III	V	0	III	V	0	0	V	0	0	IV	0	0
	II	IV	0	0	0	0	0	0	0	0	0	0	0	0	0
2	III	IV	IV	III	IV	0	III	III	0	III	III	0	0	0	0
3	IV	IV	0	IV	IV	0	IV	IV	0	IV	III	0	III	I	0

TABLE 7.—*(Continued.)—Reproduction in presence of various amounts of tartaric acid (2–5 per cent)*

Organism	2%			3%		
	Mic.	Mac.	Chl.	Mic.	Mac.	Chl.
F. aquaeductuum pusilum	0–II	0	0	0	0	0
F. asclerotium	0	0	0	0	0	0
F. aurantiacum	0	0	0	0	0	0
F. batatatis 2	II	0	0	0	0	0
F. bulbigenum	0	0	0	0	0	0
F. hyperoxysporum 1	0	0	0	0	0	0
F. javanicum 2	0	0	0	0	0	0
F. loncheceras	0	0	0	0	0	0
F. lutulatum I	0	0	0	0	0	0
F. lutulatum II	0	0	0	0	0	0
F. lycopersici 2	0	0	0	0	0	0
F. martii 2	II	0	0	0	0	0
F. moniliforme 1	III	0	0	0	0	0
F. moniliforme 2	II	0	0	0	0	0
F. moniliforme 5	0	0	0	0	0	0
F. moniliforme erumpens I	II	0	0	0	0	0
F. moniliforme erumpens II	IV	0	0	II	0	0
F. moniliforme minus	II	0	0	0	0	0
F. moniliforme subglutinans	0	0	0	0	0	0
F. neoceras	I	0	0	0	0	0
F. orthoceras triseptatum	0	0	0	0	0	0
F. ossicolum 1	III	0	0	0	0	0
F. oxysporum 2	0	0	0	0	0	0
F. rhizochromatistes	0	0	0	0	0	0
F. sclerostromaton	0	0	0	0	0	0
F. semitectum 1–VIII	II	I	0	0	0	0
F. spinaceae 2	0	0	0	0	0	0
F. trichothecioides 6	0	0	0	0	0	0
F. viride 2	0	0	0	0	0	0
F. zonatum 3	0	0	0	0	0	0

GROWTH IN PRESENCE OF VARIOUS AMOUNTS OF MALACHITE GREEN

The toxicity of aniline dyes has been commonly known for quite a long time and their use in medicine has reached an important stage of development. Malachite green[11] has been used both in this country and in Europe with different degrees of effectiveness in the control of micro-organisms.

About 50 different dyes and indicators were tested to find one that would yield the best differential growth. The dyes which have given such excellent results in medicine, such as gentian violet, acroflavin, acroviolet, and some others, failed to exert a marked inhibitory effect when used in as high concentrations as 0.1 per cent. Malachite green, on the other hand, although

[11] After this paper was submitted to the Editor the writer saw a paper by G. H. Coons (Plant pathology and physiology in relation to man, Mayo Foundation Lectures 1926–27. Some aspects of the Fusarium problem, p. 43–92) in which malachite green is shown to exert a decidedly toxic influence upon a number of Fusarium species. 1: 40,000 malachite green was the most dilute solution used.

unable to retard bacterial growth, exerted a remarkable effect upon Fusaria even in extremely dilute quantities.

Six different concentrations of malachite green, varying from 0.0002 per cent to 0.01 per cent, have been used in these experiments. The nutrient solution to which the dye was added was prepared according to the following formula: Potassium nitrate, 2 grams; Dihydrogen potassium phosphate, 1 gram; Magnesium sulphate, 1 gram; Dextrose, 10 grams; Distilled water, 1,000 c.c. Unless otherwise stated, this solution has been used in all of the remaining experiments.

Readings were made two weeks after the transfer of the inoculum into the solutions and the results are presented in the following table:

Eleven organisms failed to grow when malachite green was present in such minute concentrations as 0.0002 per cent and a number of others made only a poor growth. In the presence of 0.0005 per cent of this dye, the number of organisms which failed to grow was increased to more than forty, while many others grew very slightly or sporadically. When the concentration of malachite green was increased to 0.004 per cent, only twenty-eight organisms made any sort of growth, most of them sporadic and hardly discernible. Only three organisms were able, occasionally, to withstand the influence of a concentration of 0.01 per cent of this dye.

Some striking dissimilarities are to be noticed in the reaction of strains of the same species towards the effect of malachite green. *F. culmorum* 1–II makes no growth in the presence of 0.0005 per cent malachite green, while no. 6–II tolerates as much as 0.004 per cent. The other strains of this species show more or less pronounced differences. One can not but wonder if such sharp dissimilarities do not indicate that the specific characters whereby the members of this group are thrown together, may not be rather vague or imperfect. *F. moniliforme* 3 is the only member of this species that is highly sensitive to malachite green. *F. semitectum* 1–VIII is the only one of the nineteen strains of this species able to tolerate 0.002 per cent of the dye, while no. 1–IX and 1–XIV are the only ones unable to grow in the presence of 0.0002 per cent of malachite green. *F. trichothecioides* 3–I and 7–II do not grow when there is more than 0.0002 per cent of the dye present, whereas *F. trichothecioides* 2–II and 7–I grow even when the concentration is increased to 0.002 per cent. *F. tricinctum* 2 seems to have more tolerance for the dye than the eight strains of *F. tricinctum* 1.

Generally speaking, however, there is more similarity of reaction toward malachite green than there is of dissimilarity, as even a casual glance at the foregoing table will reveal. Nevertheless, the same lack of uniformity which has characterized the reactions of Fusaria toward other environmental factors is to be noted in the following graphical matter:

TABLE 8.—*Growth in presence of various concentrations of Malachite Green (2 weeks at 25° C.)*

Organism	0.0002%	0.0005%	0.001%	0.002%	0.004%	0.01%
F. acuminatum 1	V–V	0–0–V–0	0–0	0–0	0–0	0–0
F. acuminatum 2–I	III–IV–III–IV	0–0–0–0	0–0	0–0	0–0	0–0
F. acuminatum 2–II	II–0–III–IV	0–0–0–0	0–0	0–0	0–0	0–0
F. acuminatum 2–III	IV–IV–IV–V	IV–0–III–IV	0–0	0–0	0–0	0–0
F. acuminatum 2–IV	IV–V–V–V	0–II–0–0	0–I–0	0–0	0–0	0
F. acuminatum 2–V	IV–V–III–IV	0–0–III–III	0–0	0–0	0–0	0
F. acuminatum 2–VI	V–V–IV–IV	I–I–II–III	0–0	0–0	0–0	0
F. acuminatum 2–VII	III–IV–IV–IV	II–IV–IV–IV	IV–I–0	I–0–0	0–0	0
F. acuminatum 2–VIII	IV–IV–V–IV	II–0–IV–IV	0–0	0–0	0–0	0
F. alluviale	V	V–V	III–III	0–0	0	0
F. anthophilum	0–I–I	0–0–0–0	0–0	0–0	0–0	0
F. aquaeductuum pusilum	V–V	V–V	0–III	0–0	0–0	0
F. aquaeductuum volutum	V	IV–IV	I–III	0–II–0	0–0	0
F. arcuatum	0–0	0–0–0	0–0	0–0	0–0	0
F. asclerotium	V	V–V	IV–IV	III–IV	III–0–0	0–0
F. aurantiacum	V	V–V	IV–IV	II–I	0–0	0
F. avenaceum	III–IV	I–0	0–0	0–0	0–0	0
F. batatatis 1	V	V	III–IV	0–0	0–0	0
F. batatatis 2	V	V	III–III	0–0	0–0	0
F. bulbigenum	V	V	IV–IV	III–I	III–0–0	0
F. bullatum I	III	II	0–0	0–0	0–0	0
F. bullatum II	IV	III	II–0–0	0–0	0–0	0
F. bullatum brevius	I–0–III–III	I–0–I–I	0–0	0–0	0–0	0
F. camptoceras I	IV	III	II–II	I–I	0–0	0
F. camptoceras II	V	V	II–0–III	II–0–II	I–0–II	I–0
F. caudatum	IV	III	0–I–0–0	0–0	0–0	0
F. chlamydosporum I	I–I	0–0	0–0	0–0	0–0	0
F. chlamydosporum II	II–II	0–0	0–0	0–0	0–0	0
F. chlamydosporum III	I–I	0–0	0–0	0–0	0–0	0
F. chenopodinum	III–V	0–0	0–0	0–0	0–0	0
F. coeruleum 1–I	III–II	II	0–0	0–0	0–0	0
F. coeruleum 1–II	V	V	0–0	0–0	0–0	0
F. coerulum 2	V–V	0–II	0–0	0–0	0–0	0
F. coeruleum 3	II–II	0–0–0	0–0	0–0	0–0	0
F. commutatum	II–II	0–0	0–0	0–0	0–0	0

TABLE 8.—(*Continued*)

Organism	0.0002%	0.0005%	0.001%	0.002%	0.004%	0.01%
F. conglutinans	V	V	III–IV	0–III	0–0–III	0–0
F. cubense 1	V	V	IV–III	III–0–III	II–0–0	0–0
F. cubense 2	III–IV	I–0	0–0	0–0	0–0	0
F. culmorum 1–I	V–V	II–0	I–0–0	0–0	0–0	0
F. culmorum 1–II	V–V	0–0	0–0	0–0	0–0	0
F. culmorum 2	V	V	IV–IV	0–IV–0	0–I–0	0–0
F. culmorum 3–I	IV–IV–V–V	IV–0–V–V	III–III	II–III	0–0	0
F. culmorum 3–II	V–V–IV–V	V–0–IV–IV	III–I–0	0–0–0	0–0	0
F. culmorum 4–I	V	IV	III–IV	0–0	0–0	0
F. culmorum 4–II	V	IV	0–II–II	0–II–0	0–I–0	0–0
F. culmorum 5	V–V	0–IV–IV	II–0–0	0–0	0–0	0
F. culmorum 6–I	V	V	III–IV	III–III	0–0	0
F. culmorum 6–II	V	V	IV–IV	IV–IV	II–II	0–0
F. decemcellulare	V	IV	I–III–III	I–II–II	0–0	0
F. dimerum	V	V	V–V	IV–V	I–IV–0	0–0
F. diversisporum	V–V	0–0	0–0	0–0	0–0	0
F. ensiforme	0–0–0–0	0–0–0–0	0–0	0–0	0–0	0
F. eumartii 1–I	V	V	II–II	0–I–0	0–0	0
F. eumartii 1–II	V	V	I–IV	0–II–II	0–0	0
F. eumartii 2–I	V	IV	I–I	0–0	0–0	0
F. eumartii 2–II	V	III	0–0	0–0	0–0	0
F. eumartii 3	V–V	II–0	0–0	0–0	0–0	0
F. euoxysporum	V	V	III–IV	II–III	0–0	0
F. falcatum	IV–IV–IV	III–0–0	0–0	0–0	0–0	0
F. filiferum I	V	V	II–II	0–0	0–0	0
F. filiferum II	V	V	I–0–0	I–0–0	0–0	0
F. flocciferum	V–V	V–0	0–0	0–0	0–0	0
F. fructigenum	0–0–0–0	0–0–0–0	0–0	0–0	0–0	0
F. fructigenum maius 1	V	V	0–I–I	0–0	0–0	0
F. fructigenum maius 2–I	IV–V–V	III–IV–V	II–II	I–II	0–0	0
F. fructigenum maius 2–II	V–V	III–IV	III–III	II–II	0–0	0
F. fructigenum maius 2–III	V–V	IV–II	I–I	0–0	0–0	0
F. fructigenum maius 2–IV	IV–V	IV–III	III–III	II–II	0–0	0
F. fructigenum maius 2–V	V–V	II–V	0–0	0–0	0–0	0
F. fructigenum maius 2–VII	V–V	II–II	0–0	0–0	0–0	0

TABLE 8.—(*Continued*)

Organism	0.0002%	0.0005%	0.001%	0.002%	0.004%	0.01%
F. fructigenum maius 2–VIII	V–V	II–IV	0–0–I	0–0	0–0	0
F. fructigenum maius 3–I	V	V	III–I–IV	I–0–0	0–0	0
F. fructigenum maius 3–II	IV	III	II–III	0–0	0–0	0
F. fructigenum maius 3–III	V–V	IV–0	III–IV	0–0	0–0	0
F. gibbosum	IV	III	0–0	0–0	0–0	0
F. graminum 1	V	V	III–II	II–0	0–0	0
F. graminum 2–I	III–IV	0–0	0–0	0–0	0–0	0
F. graminum 2–II	IV–IV	0–0	0–0	0–0	0–0	0
F. herbarum 1	IV	III	III–I	0–0	0–0	0
F. herbarum 2	V–V	II–0	0–0	0–0	0–0	0
F. herbarum gibberalloides I	IV–IV	II–0	0–0	0–0	0–0	0
F. herbarum gibberalloides II	IV–IV–IV	II–III–III	0–0	0–0	0–0	0
F. herbarum pirinum I	0–I–0–0–0	0–0–0–0	0–0	0–0	0–0	0
F. herbarum pirinum II	0–0–0–0–0	0–0–0–0	0–0	0–0	0–0	0
F. hyperoxysporum 1	V	V	IV–III	III–0	III–0	0
F. hyperoxysporum 2	V–V–V	V–IV–V	III–IV	0–0	0–0	0
F. incarnatum I	V	IV	I–0–0	0–0	0–0	0
F. incarnatum II	V–IV	V–0	0–0	0–0	0–0	0
F. incarnatum IV	IV	III	II–0–0	0–0	0–0	0
F. incarnatum V	IV	III	0–II–0	0–0	0–0	0
F. javanicum 1	V	V	IV–V	III–III	0–0	0
F. javanicum 2	IV	III	III–III	III–I	0–0	0
F. lateritium I	0–0–0–0	0–0–0–0	0–0	0–0	0–0	0
F. lateritium II	0–0–0–0	0–0–0–0	0–0	0–0	0–0	0
F. lini	V	V	III–III	II–0–0	0–0	0
F. lolii	0–0	0–0–0	0–0	0–0	0–0	0
F. loncheceras	V	V	III–II	II–0	I–0–0	0–0
F. loncheceras microsporum	V	V	II–IV	0–0	0–0	0
F. longipes	0–II–III–III	0–0–0–0	0–0	0–0	0–0	0
F. lutulatum I	IV	III	II–III	I–I	0–0	0
F. lutulatum II	IV	IV	IV–III	III–II	0–0	0
F. lycopersici 1	V	IV	II–II	0–0	0–0	0
F. lycopersici 2	V	V	II–I	0–0	0–0	0
F. malli	IV	III	II–II	0–I	0–0	0
F. macroceras	V	V	III–II	0–0	0–0	0

TABLE 8.—(*Continued*)

Organism	0.0002%	0.0005%	0.001%	0.002%	0.004%	0.01%
F. martii 1	V	V	V–V–0	IV–V–0	II–III–0	0
F. martii 2	V	V	III–III	III–0–V	0–0	0
F. martii 3–I	V	V	IV–IV	III–II	0–I–0	0
F. martii 3–II	V	V	IV–IV	III–II	0–0	0
F. martii minus	V–IV–V	V–IV–IV	IV–III	0–0	0–0	0
F. martii phaseoli 1–I	IV	IV	III–IV	0–III–0	0–III–0	0–II
F. martii phaseoli 1–II	V	V	IV–IV	III–III	0–I–0–0	0–0
F. martii phaseoli 1–III	IV	III	I–II	0–I–II	0–0	0
F. martii phaseoli 2–I	V–V–V	IV–V–V	I–I	0–0	0–0	0
F. martii phaseoli 2–II	V–V	II–IV–IV	I–I	0–0	0–0	0
F. martii pisi	IV	III	II–III	I–II	0–0	0
F. martii viride	V	V	III–V	III–IV	0–III–0	0–0
F. moniliforme 1	IV–IV–IV	III–IV–III	III–IV	0–III–I	0–0	0
F. moniliforme 2	V	V	IV–IV	II–III	0–II	0–0
F. moniliforme 3	0–0–I–II	0–0–0–0	0–0	0–0	0–0	0
F. moniliforme 4	V	V	III–III	0–0	0–0	0
F. moniliforme 5	IV	III	III–III	I–I	0–0	0
F. moniliforme erumpens I	V	V	III–IV	III–IV	0–0	0
F. moniliforme erumpens II	V	V	IV–IV	III–III	0–0	0
F. moniliforme minus	V–V–V	IV–IV–IV	III–IV	III–IV	0–III–0	0–0
F. moniliforme subglutinans	III–IV–III	III–II–III	0–II–0	0–0	0–0	0
F. neoceras	IV	III	I–I	0–0	0–0	0
F. niveum 1	V	V	0–III–0	0–0	0–0	0
F. niveum 2	V–V	III–0	0–II–0	0–0	0–0	0
F. orthoceras	V	V	III–III	II–I	0–0	0
F. orthoceras triseptatum	V	V	III–III	II–II	I–0–0	0–0
F. ossicolum 1	II–I	0–I–0–0	0–0	0–0	0–0	0
F. ossicolum 2	IV	III	III–III	0–0	0–0	0
F. oxysporum 1	IV	III	III–II	0–0	0–0	0
F. oxysporum 2	V	V	II–III	0–0	0–0	0
F. oxysporum nicotianae	V	V	II–IV	0–I–III	0–0	0
F. paspalicola	V	V	0–0	0–0	0–0	0
F. pirinum	III–IV–IV	II–III–IV	0–I–0	0–0	0–0	0
F. poae I	IV–V	II–III–III	0–0	0–0	0–0	0
F. poae II	V–V	IV–V–0–IV–V	0–0	0–0	0–0	0

TABLE 8.—(*Continued*)

Organism	0.0002%	0.0005%	0.001%	0.002%	0.004%	0.01%
F. polymorphum	III–II	0–0	0–0	0–0	0–0	0
F. pusillum	V	V	IV–IV	III–IV	II–0	0–0
F. radicicola	V	V	III–IV	0–III–V	0–I–0	0–0
F. rhizochromatistes	V	V	II–III	II–0–0	0–0	0
F. rhizochromatistes-microsclerotium	V	V	III–IV	II–III	0–0	0
F. sambucinum 1	0–0	0–0	0–0	0–0	0–0	0
F. sambucinum 2	IV–V–IV–IV	IV–III–III	III–III–0	0–0	0–0	0
F. scirpi	II–III	0–0	0–0	0–0	0–0	0
F. sclerostromaton	V	V	0–III–III	0–II–II	0–I–0	0
F. semitectum 1–I	0–I–II–II	0–0–0–0	0–0	0–0	0–0	0
F. semitectum 1–II	II–II	0–0	0–0	0–0	0–0	0
F. semitectum 1–III	III–IV	0–I	0–0	0–0	0–0	0
F. semitectum 1–IV	IV–IV	0–0	0–0	0–0	0–0	0
F. semitestum 1–V	V–V	0–0	0–0	0–0	0–0	0
F. semitectum 1–VI	V–V	I–I	0–0	0–0	0–0	0
F. semitectum 1–VII	II–II	0–0	0–0	0–0	0–0	0
F. semitectum 1–VIII	V–V	IV–V	III–III	II–II	0–I–0	0
F. semitectum 1–IX	0–0–I–I	0–0–0–0	0–0	0–0	0–0	0
F. semitectum 1–X	V–V	0–0	0–0	0–0	0–0	0
F. semitectum 1–XI	0–I–II–III	0–0–0–0	0–0	0–0	0–0	0
F. semitectum 1–XII	II–II	0–0	0–0	0–0	0–0	0
F. semitectum 1–XIII	III–IV	0–0	0–0	0–0	0–0	0
F. semitectum 1–XIV	0–0–0–0	0–0–0–0	0–0	0–0	0–0	0
F. semitectum 1–XV	II–II	0–0	0–0	0–0	0–0	0
F. semitectum 1–XVI	II–II	0–0	0–0	0–0	0–0	0
F. semitectum 1–XVII	V–V	0–II	0–0	0–0	0–0	0
F. semitectum 1–XVIII	IV–V	0–IV	0–0	0–0	0–0	0
F. semitectum 2	0–0–III–III	0–0–I–I	0–0	0–0	0–0	0
F. solani 1	V	V	III–V	0–V–0–V	0–IV–0	0–0
F. solani 2–I	V	V	III–III	I–II	0–0	0
F. solani 2–II	V	V	IV–V	III–V	III–IV	0–0
F. solani 3–I	V	V	III–IV	III–IV	0–0	0
F. solani 3–II	V	V	IV–IV	III–III	0–0	0
F. solani 3–III	V	V	IV–IV	III–III	0–0	0

TABLE 8.—(*Continued*)

Organism	0.0002%	0.0005%	0.001%	0.002%	0.004%	0.01%
F. solani 4–I	V	V	IV–IV	II–IV	0–0	0
F. solani 4–II	V	V	IV–I	II–IV–V	0–0	0
F. solani minus 1–I	V	V	IV–IV	III–II	II–II	0–0
F. solani minus 1–II	V	V	IV–III	II–0–0	0–0	0
F. solani minus 2–I	V	V	IV–V	IV–IV	III–III–0	III–0
F. solani minus 2–II	V	V	V–IV	IV–IV	0–0	0
F. solani suffuscum	V	V	II–IV	0–0	0–0	0
F. spinaceae 1	V	V	III–III	III–II	0–0	0
F. spinaceae 2	V	V	III–IV	II–II	0–0	0
F. striatum 1–I	V	V	IV–III	II–0–0	0–0	0
F. striatum 1–II	V	V	II–IV	II–III	0–0	0
F. striatum 2	V	V	III–V	III–V	II–III	0–0
F. striatum 3–I	V	V	IV–IV	IV–IV	0–III–IV	0–0
F. striatum 3–II	V	V	IV–V	IV–IV	0–0	0
F. sulphureum 1	V	V	I–0–V	0–0	0–0	0
F. sulphureum 2	II–II	I–0	I–0–0	0–0–0	0–0	0
F. sulphureum 3	IV	III	II–III	I–I	0–0	0
F. theobrome	V	V	III–III	0–0	0–0	0
F. tracheiphilum	IV	III	II–II	0–0	0–0	0
F. trichothecioides 1	0–0–III–IV	0–0–I–0	0–0	0–0	0–0	0
F. trichothecioides 2–I	V	V	IV–IV	III–0	0–0	0
F. trichothecioides 2–II	IV	III	III–III	III–III	0–0	0
F. trichothecioides 2–III	IV	III	II–III	0–0	0–0	0
F. trichothecioides 3–I	V–V	0–0	0–0	0–0	0–0	0
F. trichothecioides 3–II	V–V	III–II	0–II–0	0–0	0–0	0
F. trichothecioides 4–I	IV–IV	IV–III	III–0–III	I–0–II	0–0–0	0
F. trichothecioides 4–II	III–V	0–III–V	II–0–0	0–0	0–0	0
F. trichothecioides 5	IV–V–V	III–III–III	III–II	0–0	0–0	0
F. trichothecioides 6	V	IV	II–II	0–0	0–0	0
F. trichothecioides 7–I	V	V	IV–IV	0–IV–IV	0–0	0
F. trichothecioides 7–II	I–I–I–I	0–0–0–0	0–0	0–0	0–0	0
F. tricinctum 1–II	III–II	0	0–0	0–0	0–0	0
F. tricinctum 1–III	III–III	I	0–0	0–0	0–0	0
F. tricinctum 1–IV	III–IV	I	0–0	0–0	0–0	0
F. tricinctum 1–V	III–IV	I	0–0	0–0	0–0	0

TABLE 8.—(*Continued*)

Organism	0.0002%	0.0005%	0.001%	0.002%	0.004%	0.01%
ricinetum 1–VI	I–I–II–II	0–0–0	0–0	0–0	0–0	0
ricinetum 1–VII	IV–IV	I	0–0	0–0	0–0	0
ricinetum 1–VIII	II–II	I	0–0	0–0	0–0	0
ricinetum 1–IX	III–IV	I	0–0	0–0	0–0	0
ricinetum 2	III–III–III	II–III–III	0–0	0–0	0–0	0
ncinatum	III–III	I–I–I	0–I–0	0–0–0	0–0	0
rticearum I	IV–IV–III	III–III–III	0–II–0–0	0–0	0–0	0
rticearum II	IV–IV–III	III–III–III	0–0–0	0–0	0–0	0
usinfectum	V	V	III–IV	0–0	0–0	0
ride 1	IV	IV	III–IV	III–III	0–0	0
ride 2	V	V	III–III	III–II	0–0	0
ticola	III–III–III	II–II–II	0–0	0–0	0–0	0
natum 1	V	V	III–III	0–0	0–0	0
natum 2	IV	II	II–0–0	I–0–0	0–0	0
natum 3	V	IV	IV–III	III–II	0–0	0

des → 0.001 per cent malachite green = 0
III → agar → 0.001 per cent malachite green = V
IV
III

tium → 0.004 per cent malachite green = 0 → nutrient solution = 0
III
0

um II → 0.001 per cent malachite green = II
0
0 → nutrient solution = 0

ceras II → 0.004 per cent malachite green = II → agar → 0.004 per cent = 0
0
0

tinans → 0.004 per cent malachite green = 0
0 → nutrient solution = V → agar → 0.004 per cent malachite green =
III

II → agar → 0.004 per cent malachite green = 0
II 0
0 → nutrient solution = V → agar → 0.004 per cent malachite green = 0
0

e 1 → 0.004 per cent malachite green = II
0
0 → nutrient solution = V → agar → 0.004 per cent malachite green = 0
0

rum 3–I → 0.0005 per cent malachite green = IV
0 → nutrient solution = V → 0.0005 per cent malachite green = III
V
V
V → agar → 0.0005 per cent malachite green = V
V

rum 3–II → 0.0001 per cent malachite green = V
0 → nutrient solution = 0
IV
IV

merum → 0.004 per cent malachite green = I IV
0 → nutrient solution = V → agar → 0.004 per cent malachite green = V V III

uctigenum maius 2–VIII → 0.001 per cent malachite green = 0 0
0 → nutrient solution = III → agar → 0.001 per cent malachite green = 0 0 I

uctigenum maius 3–III → 0.0005 per cent malachite green = IV 0 → nutrient solution = III → agar → 0.0005 per cent malachite green = V V III

arnatum IV → 0.001 per cent malachite green = II 0
0 → nutrient solution = 0

arnatum V → 0.001 per cent malachite green = 0 II
0 → nutrient solution = 0

rtii 1 → 0.001 per cent malachite green = V V
0 → nutrient solution = IV → agar → 0.001 per cent malachite green = 0 0 0

rtii 2 → 0.002 per cent malachite green = III 0
V → agar → 0.002 per cent malachite green = III III III

rtii phaseoli 1–I → 0.002 per cent malachite green = 0 III
0 → nutrient solution = II → agar → 0.002 per cent malachite green = 0 0 0

tii viride → 0.004 per cent malachite green = 0 III
0 → nutrient solution = 0

iliforme minus → 0.004 per cent malachite green = 0 III
0 → nutrient solution = III → agar → 0.004 per cent malachite green = 0 0 0

ceras triseptatum → 0.004 per cent malachite green = I 0 → nutrient solution = V → agar → 0.004 per cent malachite green = 0 0 0

porum nicotianae → 0.002 per cent malachite green = 0 I III → agar → 0.002 per cent malachite green = III III III

i → 0.002 per cent malachite green = 0 V 0 → nutrient solution = V → agar → 0.002 per cent malachite green = V III V → agar → 0.002 per cent malachite green = V V V

i minus 2–I → 0.004 per cent malachite green = III III 0 → nutrient solution = 0

i 4–II → 0.002 per cent malachite green = II IV V → agar → 0.002 per cent malachite green = II II I

ucinum 2 → 0.0005 per cent malachite green = III III 0 → nutrient solution = V → agar → 0.0005 per cent malachite green = 0 0 II → agar → 0.0005 per cent malachite green =

actum IV → 0.001 per cent malachite green = 0 → nutrient solution = 0 0 0

othecioides 4–II → 0.001 per cent malachite green = II 0 → nutrient solution = 0

Growth in Presence of Mercurochrome and Tannin

Two concentrations of mercurochrome (.1 and 0.2 per cent) and two concentrations of tannin (0.25 and 0.5 per cent) were used in these experiments. In order to eliminate precipitation, the nutrient solution for the mercurochrome tests was modified by replacing dihydrogen potassium phosphate with the same quantity of sodium nucleinate and by adding the dye after the solution was sterilized. Plugged, sterilized test-tubes were then filled by means of sterile 2 c.c. pipettes.

Readings were made two weeks after the transfer of the inoculum and the results are shown in the following table:

It can be seen that most of the organisms can tolerate comparatively large amounts of mercurochrome and tannic acid. Nevertheless, the same lack of uniformity of individual reactions, the same dissimilarity of different strains of a given species in their reactions towards a given environmental factor are to be witnessed here. *F. culmorum* 4–II and 6–I can grow in the presence of 0.2 per cent mercurochrome, while the remaining strains can not, just as nos. 3–I, 4–I and 6–I tolerate 0.5 per cent tannic acid whereas all others do not. *F. fructigenum maius* 3–I and 3–II are the only ones of the eleven strains of this species that can not tolerate 0.5 per cent tannic acid, and *F. fructigenum maius* 3–II can not tolerate even 0.25 per cent tannic acid. *F. incarnatum* II and V can not grow in the presence of 0.1 per cent mercurochrome, while nos. I and IV can. *F. martii phaseoli* 1–I, 1–III and 2–II show no sign of growth when there is 0.25 per cent tannic acid in the nutrient solution, while all other strains of *F. martii* and its varieties grow very well in such a solution. *F. moniliforme* 3 again shows a sharp departure from the other 8 strains by failing to grow in the presence of tannic acid. Such constant departures indicate that this strain may be an entirely different species. *F. niveum* 1 grows in the presence of 0.5 per cent tannic acid, while no. 2 does not. *F. ossicolum* 1, unlike no. 2, does not tolerate either mercurochrome or tannic acid. The strains of *F. semitectum* are extremely dissimilar in their reactions. *F. semitectum* 1–VIII, 1–X, 1–XVII, and 1–XVIII are the only ones capable of growing in the presence of 0.5 per cent tannic acid, while 1–II, 1–V, 1–VI, 1–VII, 1–IX, 1–XI, 1–XII, 1–XIV, 1–XV, 1–XVI and *F. semitectum* 2 can not tolerate even 0.25 per cent of the acid. *F. solani* 2–I is the only member of the solani group capable of growing very readily in the presence of 0.5 per cent tannic acid, while all others either do not grow at all, or grow very slightly or occasionally. Similarly, *F. striatum* 2 gives positive results in 0.5 per cent tannic acid while no. 1–II gives negative results. Certain members of the *F. trichothecioides* group exhibit a similar departure from the common path. On the other hand, the strains of *F. tricinctum* are remarkably uniform in

TABLE 9.—*Growth in presence of mercurochrome and tannic acid (2 weeks at 25° C.)*

Organism	Mercurochrome		Tannic acid	
	0.1%	0.2%	0.25%	0.5%
F. acuminatum 1	II–II	I–I–I	III–II	0–0
F. acuminatum 2–I	II–II	0–0–0	I–III	0–0
F. acuminatum 2–II	II–II	0–0–0	II–III–II	0–0–0
F. acuminatum 2–III	II–II	0–II–0–0	II–III–II	0–0–0
F. acuminatum 2–IV	II–II	0–I–0–I	II–III–IV	0–0–0
F. acuminatum 2–V	III–I	0–0–0	0–II–II	0–0–0
F. acuminatum 2–VI	I–I–I	0–0–0	I–II–II	0–0–0
F. acuminatum 2–VII	I–I	0–0–0	II–I–II	0–I–0–0
F. acuminatum 2–VIII	I–I	0–0–0	0–II–IV–III	0–0–0
F. alluviale	II–II	0–0–0	III–III	0–0
F. anthophilum	0–0–0	0–0	II–II	0–0
F. aquaeductuum pusilum	0–0	0–0	0–0	0–0
F. aquaeductuum volutum	0–0	0–0–0	0–0–0	0–0–0
F. arcuatum	0–II–0–0	0–I–0–0	III–II	0–0–0
F. asclerotium	III–III	I–II–II	V–V	IV–V
F. aurantiacum	III–II	I–II–II	IV–IV	IV–IV
F. batatatis 1	II–II	0–0	III–IV	I–I
F. batatatis 2	III–III	0–I–II–II	IV–IV	III–III
F. bulbigenum	III–III	II–II–II	IV–IV	IV–III
F. bullatum I	I–0–I–0	0–0–0	III–II–III	0–I–II
F. bullatum II	II–I–0–I	0–0–0	III–III–III	I–II–II–I
F. bullatum brevius	I–0–0–0–0	0–0	III–III	II–I
F. camptoceras I	I–I–I	0–I–I–I–I	III–III–III	III–II–I
F. camptoceras II	II–II	0–I–0–0	IV–IV–III	III–II–0–0
F. caudatum	0–I–0–0	0–I–0–0–0–I	0–0	0–0
F. chlamydosporum I	II–I–0	0–0–0	0–0–0	0–0–0
F. chlamydosporum II	II–II–I	0–I–0–0	0–0–0	0–0–0
F. chlamydosporum III	I–I–0–0	0–0–0	0–0–0	0–0–0
F. chenopodinum	I–II–0	0–0	0–0	0–0
F. coeruleum 1–I	II–III–II	0–I–I	II–IV–II	0–0–0–0
F. coeruleum 1–II	I–III	0–0–I–I	0–0–IV–0	0–0–0
F. coeruleum 2	II–II	0–I–0–I	II–IV	I–0
F. coeruleum 3	I–0–0	0–0	II–0	0–0

TABLE 9.—(*Continued*)

Organism	Mercurochrome		Tannic acid	
	0.1%	0.2%	0.25%	0.5%
F. commutatum	I–I–I	0–0	0–0	0–0
F. conglutinans	II–I	0–II–0–0–0	III–IV	II–IV
F. cubense 1	II–II	0–0	IV–IV	IV–IV
F. cubense 2	II–I	0–0	IV–IV	III–III
F. culmorum 1–I	0–I–I–II	0–0–0	0–0–0	0–0–0
F. culmorum 1–II	0–I–I	0–0–0	0–0–0	0–0–0
F. culmorum 2	II–II	0–0	0–0	0–0
F. culmorum 3–I	III–III	0–0–0	III–III–IV	0–III–III–I
F. culmorum 3–II	II–I	0–0–0	II–II–II	0–0–0
F. culmorum 4–I	II–II	0–0–I	III–III–III	I–0–II–I–0
F. culmorum 4–II	III–III	0–I–II–II	III–II–II	0–0–0
F. culmorum 5	III–III	0–I–0–0	0–III–III–II	0–0–0–0
F. culmorum 6–I	III–III	III–II–II	IV–IV–IV	III–IV–IV
F. culmorum 6–II	II–II–II	0–0–0	0–0–0	0–0–0
F. decemcellulare	I–I–I	0–0–0	II–II–II	0–0–0
F. dimerum	I–I	0–0	0–0	0–0
F. ensiforme	II–0–0	0–0	0–0	0–0
F. eumartii 1–I	IV–III	I–II–II	0–0–0	0–0–0
F. eumartii 1–II	IV–III	II–II–II	0–0–III–II	0–0–0
F. eumartii 2–I	I–II–II	I–I–I	0–0–0	0–0–0
F. eumartii 2–II	I–III–II	0–I–0	0–0	0–0–0
F. eumartii 3	II–II	I–I	0–0	0–0
F. euoxysporum	III–III	II–0–I–II	III–IV	0–II–II–I
F. falcatum	0–0	0–0	0–0	0–0
F. filiferum I	II–I–I–0	0–I–0–II	II–IV–II	0–0–I
F. filiferum II	II–II–I	0–I–I	III–III–IV	I–0–0–I
F. flocciferum	0–0	0–0	0–0	0–0
F. fructigenum	I–0–0	0–0	III–III	0–0
F. fructigenum maius 1	II–0	0–0	IV–IV	II–II
F. frucigtenum maius 2–I	II–0–0	0–0	IV–IV	II–III
F. fructigenum maius 2–II	0–I	0–0	IV–IV	III–III
F. fructigenum maius 2–III	I–I	0–I–0–0	IV–IV–III	0–I–III–0–I
F. fructigenum maius 2–IV	II–II	0–0–0	IV–IV–IV	III–IV–III

TABLE 9.—(*Continued*)

Organism	Mercurochrome		Tannic acid	
	0.1%	0.2%	0.25%	0.5%
F. fructigenum maius 2–V	I–I	0–0–0	III–III–III	III–II–II
F. fructigenum maius 2–VII	I–I	0–0–I	III–III–III	0–I–II–0
F. fructigenum maius 2–VIII	I–I	0–I–0	III–IV–IV	0–III–0
F. fructigenum maius 3–I	I–I	0–I–0–0	0–III–II–0	0–0–0
F. fructigenum maius 3–II	I–0	0–0	0–0	0–0
F. fructigenum maius 3–III	I–II–I	0–0–0	II–III–III–IV	0–I–0–0–I
F. gibbosum	II–0–0	0–I–0–0	II–III	0–0
F. graminum 1	I–0–I	0–0	II–III	0–0
F. graminum 2–I	0–0	0–0	IV–IV	0–I–0
F. graminum 2–II	II–0–0	I–0–0	III–IV–IV	III–III
F. herbarum 1	0–I–0–II	0–0	IV–II	III–0
F. herbarum 2	I–I–0–0	0–0	II–II	0–0
F. herbarum gibberalloides I	III–III	0–0–0	II–III–III	0–I–I–0
F. herbarum gibberalloides II	II–II	0–I–0–0–0	III–III–III	0–0
F. herbarum pirinum I	I–II–II	I–I–I	0–I–II–III	0–0–0
F. herbarum pirinum II	II–II–II	I–I–I	I–0–I–III–0	0–0–0
F. hyperoxysporum 1	III–III	II–0	IV–IV	III–II
F. hyperoxysporum 2	III–III	II–II	IV–IV	IV–III
F. incarnatum I	I–II–I	0–II–I–0	III–III–IV	III–I–II
F. incarnatum II	0–0	0–0–0	II–III	I–I
F. incarnatum IV	III–III–I	0–0–0	III–III–III	II–I–II
F. incarnatum V	0–0	0–0–0	II–II–II	0–I–0–0
F. javanicum 1	II–II	0–0	IV–IV	II–II
F. javanicum 2	II–II	I–I	IV–IV	IV–IV
F. lateritium I	I–0	0–0	I–II	0–0
F. lateritium II	I–0–I	0–0–0	0–II–II–I	0–0–0
F. lini	I–III–III	I–0–0–0–II	II–III	0–0–0
F. lolii	II–II	0–I–0	III–I	III–I
F. loncheceras	III–IV	II–0–III–III	IV–IV	IV–IV
F. loncherceras microsporum	III–II	II–I–0–I	III–IV	III–III
F. longipes	0–I–I–0	0–0	II–II	0–0

TABLE 9.—(*Continued*)

Organism	Mercurochrome		Tannic acid	
	0.1%	0.2%	0.25%	0.5%
F. lutulatum I	III–III	0–I–II–I–II	IV–IV–IV	III–III–III
F. lutulatum II	III–II	0–II–II–II	IV–IV–IV	III–IV–III
F. lycopersici 1	II–I	I–0–0–I	IV–IV	III–III
F. lycopersici 2	III–III	I–I–I	IV–IV	III–IV
F. malli	III–III	II–II	IV–IV	IV–IV
F. macroceras	II–II	0–I–I	0–I	0–0
F. martii 1	III–III	I–II–II	IV–IV	0–III–0
F. martii 2	III–III	II–II	IV–IV	III–III
F. martii 3–I	III–IV–III	0–II–I	IV–II–III	0–I–0–0
F. martii 3–II	III–IV–III	IV–I–III–II–II	II–IV–III	0–0–0
F. martii minus	III–III	II–0–III	III–IV	0–II–0
F. martii phaseoli 1–I	III–III	III–III–I	0–0–0	0–0–0
F. martii phaseoli 1–II	III–III–II–III	I–III–I–I	II–III–III	0–II–I–0
F. martii phaseoli 1–III	IV–IV	II–II	0–0–0–0	0–0–0–0
F. martii phaseoli 2–I	I–I–II	I–0–0–I	II–IV–0	0–0–0–0
F. martii phaseoli 2–II	II–IV–I	I–III–0	0–0–0	0–0–0–0
F. martii pisi	III–III	II–0	IV–IV	III–III
F. martii viride	III–III	0–II–I	IV–IV	I–III
F. moniliforme 1	II–I	0–0	III–III	III–II
F. moniliforme 2	III–III	II–I–II	III–IV	III–IV
F. moniliforme 3	II–II	0–I–I–0	0–0	0–0
F. moniliforme 4	III–II	0–I–I	III–IV	III–III
F. moniliforme 5	II–III	I–II	IV–IV	III–III
F. moniliforme erumpens I	II–III	0–I–I–I	IV–IV–IV	III–III–III
F. moniliforme erumpens II	II–III	I–I–I	IV–IV–V	III–III–IV
F. moniliforme minus	III–III	I–II	III–IV	III–III
F. moniliforme subglutinans	I–I	0–0–0	IV–IV	III–III
F. neoceras	III–I	0–I–I	IV–IV	III–III
F. niveum 1	III–II	I–I–I	IV–IV	III–III
F. niveum 2	II–I	0–0	IV–IV	0–0
F. orthoceras	III–II	0–I–II–I	IV–IV	III–III
F. orthoceras triseptatum	III–III	I–II–II	IV–IV	IV–IV
F. ossicolum 1	0–0	0–0–0	0–0–0	0–0–0

TABLE 9.—(*Continued*)

Organism	Mercurochrome		Tannic Acid	
	0.1%	0.2%	0.25%	0.5%
F. ossicolum 2	II–I	0–I–II	I–IV	0–III–II
F. oxysporum 1	III–II	II–II–II	IV–IV	III–IV
F. oxysporum 2	II–III	0–I–II	V–IV	V–IV
F. oxysporum nicotianae	III–III	II–II–II	IV–V	III–IV
F. paspalicola	III–II	I–0–I–I	II–III	0–0
F. pirinum	I–0–0	0–0	III–II	0–0
F. poae I	0–0–0–0	0–0–0	III–III–III	0–I–0–0
F. poae II	I–0–0–0	0–0–0	III–III–IV	0–I–I–0
F. polymorphum	0–0	0–0	0–0	0–0
F. pusillum	III–III	I–II–0–II	III–IV	0–0
F. radicicola	II–III	0–I–II–I	III–IV	I–II
F. rhizochromatistes	III–III	III–III	IV–IV	III–III
F. rhizochromatistes-microsclerotium	III–III	I–0–II	IV–IV	IV–IV
F. sambucinum 1	I–0–0	0–0	0–0	0–0
F. sambucinum 2	I–II–II	0–I–I	I–II	I–I
F. scirpi	III–II	I–I–I	II–0	0–0
F. sclerostromaton	II–II	I–0–I–II	IV–IV	IV–IV
F. semitectum 1–I	I–II	I–I–I	0–III–II–II	0–0–0
F. semitectum 1–II	I–I	0–I–0–I	0–0–0	0–0–0
F. semitectum 1–III	I–III	0–I–II–I	0–II–0–II	0–0–0
F. semitectum 1–IV	II–II	0–I–I–0	0–0–II–III	0–0–0
F. semitectum 1–V	I–0–0	0–0–0	0–0–0	0–0–0
F. semitectum 1–VI	I–0–0	0–I–0–0	0–0–0	0–0–0
F. semitectum 1–VII	I–I	0–I–0–0	0–0–0	0–0–0
F. semitectum 1–VIII	III–III	II–III–II	IV–IV–V	III–IV–V
F. semitectum 1–IX	II–I	0–I–I–0	0–0–0	0–0–0
F. semitectum 1–X	II–I	0–0–0	II–III–III	I–I–II
F. semitectum 1–XI	I–II	0–I–I–0	0–0–0	0–0–0
F. semitectum 1–XII	I–I	0–I–I–I	0–0–0	0–0–0
F. semitectum 1–XIII	I–0–0	0–I–0–0	0–III–0–0–0	0–I–0–0–0
F. semitectum 1–XIV	I–0–0	0–0–0	0–0–0	0–0–0
F. semitectum 1–XV	II–0–0	0–I–0–0	0–0–0	0–0–0

TABLE 9.—(*Continued*)

Organism	Mercurochrome		Tannic Acid	
	0.1%	0.2%	0.25%	0.5%
F. semitectum 1–XVI	II–I	I–I–0–0	0–0–0	0–0–0
F. semitectum 1–XVII	II–I	0–0–0	III–IV–III	I–II–I
F. semitectum 1–XVIII	I–0–I	0–0–0	III–0–I	II–0–0
F. semitectum 2	II–II	II–I	0–0–0	0–0–0
F. solani 1	III–II	0–I–I	IV–IV	0–III–0
F. solani 2–I	III–IV–IV	IV–III–IV	IV–IV–IV	III–IV–IV
F. solani 2–II	IV–V	II–II	II–IV	0–0
F. solani 3–I	III–III	I–III–I	IV–IV–III	0–I–I
F. solani 3–II	III–III	I–III–I	IV–0–IV–0	0–0–0
F. solani 3–III	IV–III	I–III–I	IV–IV–IV	0–I–0–I
F. solani 4–I	II–III	0–I–I	0–IV–IV–0	0–0–0
F. solani 4–II	III–IV	0–I–III	0–IV–V	0–0–0
F. solani minus 1–I	IV–III	0–II–I–II	III–0–IV–0	0–0–0
F. solani minus 1–II	IV–III	0–I–I–I	II–0–0	0–0
F. solani minus 2–I	III–III	I–II–II	IV–V–IV	I–0–I–II
F. solani minus 2–II	III–II	0–II–I–I	III–IV–III	0–0–0
F. solani suffuscum	II–I	0–0	II–III	0–0
F. spinaceae 1	III–II	0–II–II	IV–III	II–I
F. spinaceae 2	III–III	I–I	III–IV	II–II
F. striatum 1–I	III–III	0–II–I–II	IV–IV–V	I–I–I
F. striatum 1–II	I–I	0–0–0	I–III–III	0–0–0
F. striatum 2	I–II	0–II–0	IV–IV	III–III
F. striatum 3–I	II–III	0–I–II–I	0–0–IV–II	0–0–I–0
F. striatum 3–II	III–III	0–II–II–II	III–IV–III	0–0–0
F. sulphureum 1	I–I–I	0–0	II–II	0–I–0
F. sulphureum 2	I–II–I	0–0	0–0	0–0
F. sulphureum 3	I–II–I	0–I–I	II–II	0–0
F. theobrome	II–III	0–I–I–0	III–IV	0–0
F. tracheiphilum	III–III	0–II–II	IV–IV	III–III
F. trichothecioides 1	0–0	0–0	IV–V	IV–V
F. trichothecioides 2–I	I–II–I	0–I–0–0	0–IV–IV	0–0–0
F. trichothecioides 2–II	II–III–I	0–I–I–I	III–III–III	0–I–II
F. trichothecioides 2–III	I–I–I	0–0–0	0–II–I	0–0–0
F. trichothecioides 3–I	0–I–0–0	0–0–0	II–III–IV	II–II–II

TABLE 9.—(*Continued*)

Organism	Mercurochrome		Tannic Acid	
	0.1%	0.2%	0.25%	0.5%
F. trichothecioides 3–II	0–0–0	0–0–0	III–III–IV	0–0–0
F. trichothecioides 4–I	0–0–II–0	0–0–0–0	0–0–0	0–0–0
F. trichothecioides 4–II	I–I–I	0–0–0	II–III–III	0–I–I–0
F. trichothecioides 5	III–II	0–I–I–0	III–IV	II–III
F. trichothecioides 6	III–I–III	0–I–I–II	IV–V	III–IV
F. trichothecioides 7–I	II–II	I–I–I	0–0–IV–0	0–0–0
F. trichothecioides 7–II	0–0	0–0–0	III–III–III	II–I–0
F. tricinctum 1–II	0–0	0–0–0	I–0–II–0	0–0–0
F. tricinctum 1–III	0–0	0–0–0	0–I–II–II	0–I–0–0
F. tricinctum 1–IV	0–0	0–0	0–I–0–III	0–0–0–0
F. tricinctum 1–V	0–0	0–0–0	II–II–I	0–0–0
F. tricinctum 1–VI	0–0	0–0–0	II–II–II	0–0–0
F. tricinctum 1–VII	0–0	0–0–0	0–II–0–0	0–0–0
F. tricinctum 1–VIII	0–0	0–0–0	0–II–0–0	0–0–0
F. tricinctum 1–IX	0–0	0–0–0	I–I–II	0–0–0
F. tricinctum 2	0–0	0–0	II–II	II–I
F. uncinatum	II–II–II	I–II–II	III–III–IV	II–III–II
F. urticearum I	I–I	0–0–0	0–0–0	0–0–0
F. urticearum II	I–I	0–0–0	0–0–0	0–0–0
F. vasinfectum	II–III	0–III–II	IV–IV	IV–IV
F. viride 1	III–III	I–I–I	III–IV	II–II
F. viride 2	III–II	II–II	V–V	IV–V
F. viticola	II–I–I	0–0	II–III	II–0
F. zonatum 1	III–II	0–0–0	IV–V	II–II
F. zonatum 2	III–II	II–II	IV–III	III–III
F. zonatum 3	III–III	I–II–II	IV–IV	IV–IV

F. camptoceras II → 0.5 per cent tannin = III / II / 0

0 → nutrient solution = V → agar → 0.5 per cent tannin = 0 / 0 / 0

F. eumartii 2–II → 0.5 per cent tannin = 0

0 → nutrient solution = 0

F. herbarum pirinum II → 0.25 per cent tannin = I / 0 / III

0 → nutrient solution = 0

F. lini 1 → 0.5 per cent tannin = 0 / 0

0 → nutrient solution = 0

F. martii minus → 0.5 per cent tannin = 0 / II

0 → nutrient solution = 0

F. semitectum 1–XIII → 0.25 per cent tannin = 0 / 0

0 → nutrient solution = 0

F. solani → 0.5 per cent tannin = III / 0

0 → nutrient solution = 0

F. solani minus 1–I → 0.25 per cent tannin = III / 0 / IV

0 → nutrient solution → V → agar → 0.25 per cent tannin = V / V / III

F. solani minus 1–II → 0.25 per cent tannin = II / 0

0 → nutrient solution = V → agar → 0.25 per cent tannin = V / V / V

F. tricinctum 1–IV → 0.25 per cent tannin = 0 / I / 0

III → agar → 0.25 per cent tannin = III / II / II

their reactions towards mercurochrome and 0.5 per cent tannic acid. Other groups are also capable of exhibiting such a behavior as an examination of the foregoing table will show.

In case of a few organisms the effect of tannic acid was tested for two successive generations and it was found that tannin can act not only as an inhibitive substance, but, as the following results indicate, it seems to be toxic enough to kill some of the organisms.

GROWTH IN PRESENCE OF VARIOUS AMOUNTS OF SODIUM TRIBROMPHENATE AND SODIUM 2.4–DINITROPHENOXIDE

In their search for a suitable internal antiseptic, Bechhold and Ehrlich (1) found that the introduction of halogens into the phenol ring increased the disinfecting property of phenol to such an extent that one molecule of pentabromphenol exhibited the same action on the diphtheria bacillus as 500 molecules of phenol. An interesting fact brought to light by these two investigators was that the introduction of halogens into phenol in such proportion as to form monobromphenol, actually lowers the toxicity; but the toxic property is increased in tribromphenol, reaching its peak with tetra- and pentabromphenols, to be lowered and lost again with the higher halogen-bound substances. Archer[12] found that the sodium salt of tribromphenol gave very satisfactory results when used as a disinfectant against five species of bacteria.

A preliminary test with sodium tribromphenate gave such satisfactory results that the writer used it to obtain differential growth in Fusaria. Large numbers of phenol compounds have been tested and discarded because they lacked the ability to exert marked inhibitive action even in comparatively high concentrations. Sodium tribromphenate and sodium 2.4-dinitrophenoxide yielded the best results. Three concentrations of sodium tribromphenate (0.001,. 0.0025, and 0.005 per cent) and two concentrations of sodium dinitrophenoxide (0.01 and 0.02 per cent) have been used. The sodium salt of tetrachlor-phenol is much more powerful than sodium tribromphenate, but owing to a delay in securing this compound, the writer has not had the opportunity to test all of his cultures and incorporate the results in this paper.

Twenty strains failed to grow in the presence of 0.001 per cent sodium tribromphenate, and fifteen others made only a slight growth. Less than one-third of all the Fusaria used were able to make any sort of growth when the amount of this substance was increased to 0.005 per cent. In case of sodium dinitrophenoxide, however, comparatively few strains were totally inhibited.

[12] Archer, W. A. The comparative values of the disinfecting action of sodium tribromphenate and other chemicals. (Unpublished.)

TABLE 10.—*Growth in presence of sodium tribromphenate and sodium dinitrophenoxide (2 weeks at 25° C.)*

Organism	Tribromphenol sodium salt			Sodium 2.4 dinitrophenoxide	
	0.001%	0.0025%	0.005%	0.01%	0.02%
F. acuminatum 1		II–II	0–0–0–0	III–III–III–III	II–0–0–0
F. acuminatum 2–I	III–III–0–0–0	0–0	0–0–0	0–IV–II–0	0–0–0–0
F. acuminatum 2–II	III–IV	0–0	0–0–0	0–0–0–0	0–0–0–0
F. acuminatum 2–III	III–III	0–0	0–0–0	IV–0–0–III	0–0–0–0
F. acuminatum 2–IV	II–I	0–0	0–0–0	V–V–V–0	0–0–0–0–IV
F. acuminatum 2–V	0–0–0–0	0–0–0	0–0–0	0–II–0–III	0–0–0–0
F. acuminatum 2–VI	0–II–0–0–III	0–0	0–0–0	III–0–0–0	0–0–0–0
F. acuminatum 2–VII	II–I	0–0	0–0–0	0–0–0	0–0–0–0
F. acuminatum 2–VIII	0–0	0–0	0–0–0	0–0–0	0–0–0–0
F. alluviale		II–IV	0–0–0	V–V–V–V	V–V–V–V
F. anthophilum	III–III	0–0	0–0–0	0–II–II–I	I–0–0–0
F. aquaeductuum pusillum	IV–V	0–0	I–0	II–0–0	0–0–0–0
F. aquaeductuum volutum	0–0–0	0–0	0–0–0	I–0–0–0	0–0–0–0
F. arcuatum	II–III	0–0	0–0–0	0–0–II	0–0–0–0
F. asclerotium		IV–IV	II–0–I–I	V–IV–V	V–III–I–IV
F. aurantiacum		IV–IV	II–II–II	IV–V–I–V	V–II–0–IV
F. batatatis 1		II–II	0–0–I–I	V–V–V	II–I–V–II
F. batatatis 2		IV–V	I–I–I–I	IV–V–V–V	II–0–0–I
F. bulbigenum	III–IV	I–I–II	0–0–I–0	V–V–II–V	V–0–III–IV
F. bullatum I	IV–IV	II–I	0–0–0	0–V–V–II	0–II–0–0
F. bullatum II	V–V	II–I	0–0–0	V–III–IV–V	0–0–0–0
F. bullatum brevius		III–III	0–0–0–II	V–V–V–V	V–V–III–0–0
F. camptoceras I		III–III	0–0–I–0–0	IV–IV–IV	0–0–III–IV–III
F. camptoceras II		II–I	0–0–0–0	III–IV–0–III	0–0–IV–III–0
F. caudatum		I–0–0–0	0–0–0–0	0–0–0–II	0–0–0–0
F. chlamydosporum I	I–II	0–0–0	0–0–0	0–0–0–II	0–0–0–0
F. chlamydosporum II	II–II	0–0	0–0–0	III–III–II–II	II–0–0–II
F. chlamydosporum III	III–V	0–0	0–0–0	II–0–II–0	0–0–0–I
F. chenopodinum	II–II	0–0–0	0–0–0–0	0–0–0–III	0–0–0–III
F. coeruleum 1–I		V–V	II–II–II–IV	V–V–V–V	V–V–V–V
F. coeruleum 1–II		II–IV	0–0–0–0	V–V–V–V	V–II–I–V
F. coeruleum 2		V–V	0–0–0–0	V–V–V–V	V–V–II–IV
F. coeruleum 3	I–I	0–0	0–0–0	0–0–0–0	0–0–0–0
F. commutatum	I–I–0–0	0–0	0–0–0–0	II–I–II–0	0–0–0–I

TABLE 10.—(*Continued*)

Organism	Tribromphenol sodium salt			Sodium 2.4 dinitrophenoxide	
	0.001%	0.0025%	0.005%	0.01%	0.02%
F. conglutinans		V–V	I–II–V–IV	V–V–V–V	II–II–III–III
F. cubense 1		III–III	I–0–I–II	V–V–V–V	V–V–IV–IV
F. cubense 2		III–III	0–0–0–0	V–V–II–V	0–I–II–V
F. culmorum 1–I	II–III	0–0	0–0–0	V–II–V–0	0–0–0–0
F. culmorum 1–II	II–III	0–0	0–0–0	0–0–V	0–0–0–0
F. culmorum 2	III–IV	0–0	0–0–0	III–0–0–II	0–0–0–0
F. culmorum 3–I	III–IV	II–II–0	0–0–0–0	0–V–III–III	I–0–0–III
F. culmorum 3–II	0–0–0–0	0–0–0	0–0–0	III–0–0–0	0–0–0–0
F. culmorum 4–I		I–III	0–0–0–0	IV–III–III–0	0–0–0–0
F. culmorum 4–II		III–III	0–0–0–0	IV–IV–III–V	0–0–III–II
F. culmorum 5	I–I	0–0	0–0–0	0–V–II–0	0–0–0–0
F. culmorum 6–I		V–V	0–0–II–II	III–V–V–V	0–0–II–V
F. culmorum 6–II	III–III	0–II–0	0–0–0–0	IV–V–I–V	0–0–0–I
F. decemcellulare		II–III	0–0–0–0	V–0–V–V–V	I–0–0–I
F. dimerum	0–0	0–0	0–0–0	III–0–0–II	I–0–0–II
F. diversisporum	II–I	0–0	0–0–0–0	V–V–III–0	I–0–0–II
F. ensiforme	0–0–I–I	0–0	0–0–0	II–II–II	I–I–0–0
F. eumartii 1–I	0–0	0–0	0–I–0–0	V–IV–V–V	II–II–II–II
F. eumartii 1–II		IV–IV	I–0–0–I	V–V–V–V	V–II–I–IV
F. eumartii 2–I	V–V	IV–0	0–0	III–V–V	0–0–II–V
F. eumartii 2–II	I–I	0–0	0–0–0	0–0–V–V	II–I–III–II
F. eumartii 3	I–I	0–0	0–0–0	0–III–V–0	0–0–0–0
F. euoxysporum		III–IV	0–0–0–0	V–V–V	0–0–II–0
F. falcatum	I–0	0–0	0–0–0	II–0–0–II	0–0–0–0
F. filiferum I	IV–IV	0–V	0–0–0–0	IV–III–V	0–0–0–0
F. filiferum II	IV–IV	I–III	0–0–0–0	IV–IV–III	0–0–I–0
F. flocciferum	V–V	0–0	0–0–0	0–0–II–0	V–V–I
F. fructigenum	II–III	0–0	0–0–0–0	0–0–0	0–0–0
F. fructigenum maius 1	V–V	0–0	0–0–0	III–V–V	IV–IV–V
F. fructigenum maius 2–I	V–V	I–I	II–0–0–II	III–IV–0–V	0–0–III–V
F. fructigenum maius 2–II	IV–IV	II–II	0–0–0	II–V–V	IV–V–0–V
F. fructigenum maius 2–III	II–III	0–0	0–0–0	V–V–V	V–III–III–III
F. fructigenum maius 2–IV	IV–IV	I–II	0–0–0–0	V–IV–V	IV–V–0–V
F. fructigenum maius 2–V	III–IV	I–I	0–0–0	III–III–III	0–0–III–0

TABLE 10.—(*Continued*)

Organism	Tribromphenol sodium salt			Sodium 2.4 dinitrophenoxide	
	0.001%	0.0025%	0.005%	0.01%	0.02%
F. fructigenum maius 2–VII	IV–II	0–0	0–0–0–0	V–V–V	II–0–0–III
F. fructigenum maius 2–VIII	III–IV	I–II	0–0–0–0	V–III–V–III	III–0–0–III
F. fructigenum maius 3–I	II–0–I–II	0–0	0–0–0	V–IV–IV–0	0–0–0–0
F. fructigenum maius 3–II	III–0–I–II	0–0	0–0–0	0–0–0–0	0–0–0–0
F. fructigenum maius 3–III	IV–V–III–III	0–0	0–0–0	0–0–V–0	0–0–0–0
F. graminum 1	V–V	0–0	0–0–0–0	0–0–V–0	0–0–0–0
F. graminum 2–I	IV–IV	0–0	0–0–0	III–III–V–III	0–0–V–V
F. graminum 2–II	III–II	0–0	0–0–0	0–V–0–0	0–0–IV
F. gibbosum	IV–V	0–0	0–0–0–0	0–0–V–0	III–III–0–III
F. herbarum 1	II–III	0–0	0–0–0–0	0–0–0–0	0–0–0–0
F. herbarum 2	V–V	0–0	0–0–0–0	V–V–II–V	I–0–0–I
F. herbarum gibberalloides I	III–IV	I–I	0–0–0–0	II–II–II–II	II–0–0–I
F. herbarum gibberalloides II		II–III–IV	0–0–0	III–II–V–III	0–0–II–III
F. herbarum pirinum I	III–IV	0–0–0	0–0–0	IV–III–III–V	V–0–0–0–0
F. hebarum pirinum II	II–II	I–0–0	0–0–0–0	II–0–II–0	0–0–0–I–0
F. hyperoxysporum 1		V–V	II–IV–V–I	V–V–V	0–0–II–V
F. hyperoxysporum 2		IV–V	I–I–III–I	III–IV–V	I–II–V–IV
F. incarnatum I	III–V	I–I	0–0–0–0	IV–V–IV–V	III–I–0–II
F. incarnatum II	I–I	0–0	0–0–0–0	II–V–0–III	II–0–0–I
F. incarnatum IV	I–II	0–0	0–0–0–0	II–I–0–V	0–I–0–0
F. incarnatum V	0–0–0–0	0–0	0–0–0–0	III–III–I–II	II–0–0–I
F. javanicum 1		II–II	II–0–0–I	IV–V–V–V	I–I–0–III–V
F. javanicum 2		V–IV	II–II–0	III–V–V–V	0–0–0–III–III
F. lateritium I	I–II	I–I	0–0–0–0	II–III–II–II	0–0–0–0
F. lateritium II	II–II	0–I–0	0–0–0–0	III–III–0–III	0–0–0–0
F. lini		V–V	II–I–III	IV–IV	V–I–V–V
F. lolii	V–V	0–0	0–0–0–0	I–I–III–0	0–0–0–0
F. loncheceras		V–V	II–0–I–II	V–V–V–V	0–III–0–V
F. loncheceras microsporum	IV–IV	0–0	0–0–0	0–V–V–V	II–I–0–0
F. longipes	0–0	0–0	0–0–0	0–II–II–0	0–0–0–0
F. lutulatum I		III–III	II–I–II–II	IV–I–IV–III	IV–IV–IV–IV
F. lutulatum II		III–III	II–I–II–III	IV–II–V–V	IV–IV–III–III
F. lycopersici 1		II–II	0–0–I–0	V–V–V–IV	V–0–0–0–0
F. lycopersici 2		III–III	I–I–II–II	V–V–V–V	II–0–0–0–0

TABLE 10.—(*Continued*)

Organism	Tribromphenol sodium salt			Sodium 2.4 dinitrophenoxide	
	0.001%	0.0025%	0.005%	0.01%	0.02%
F. malli		V–V	0–0–II–0	V–V–IV–IV	0–0–IV–V
F. macroceras	III–III	I–I	0–0–0–0	III–II–II–III	0–0–0–0
F. martii 1		V–V	V–0–II–II	V–V–III	V–V–I–V
F. martii 2		IV–IV	II–II–II–II	V–V–V–V	I–V–IV–IV
F. martii 3–I		II–III	I–I–I–I	V–V–V–V	V–V–0–II
F. martii 3–II		II–IV	I–I–I–I	V–II–V–V	V–V–II–III
F. martii minus		IV–II	I–I–I–I	V–V–V–V	III–II–IV–V
F. martii phaseoli 1–I	I–IV	I–0–II–I	0–0–0–0	IV–V–V–III	0–0–II–II
F. martii phaseoli 1–II	V–V	0–0–0–0	0–I–I–I	IV–III–II–V	I–II–II–I
F. martii phaseoli 1–III	I–0–0–0	0–0–0	0–0–0	0–II–0–IV	V–V–II–I
F. martii phaseoli 2–I	V–V	0–0	0–0–0–0	III–IV–IV	0–0–I–IV
F. martii phaseoli 2–II	0–0–0–0	0–0	0–0–0–0	0–II–0–IV	0–V–0–0
F. martii pisi		III–III	I–I–II–I	III–V–V–III	II–0–IV–0
F. martii viride		V–V	II–II–I–II	V–V–V–V	V–V–V–V
F. moniliforme 1		III–III	0–0–I–I	V–V–V–V	III–I–0–0
F. moniliforme 2		IV–V	II–I–I–II	V–V–III–V	0–0–0–I
F. moniliforme 3		I–III	0–0–0–0	II–II–III–II	II–0–II–0–III
F. moniliforme 4		III–III	II–0–I–0	V–V–V–V	II–I–0–I
F. moniliforme 5		III–III	0–0–0–0	V–V–V–V	0–0–IV–I
F. moniliforme erumpens I		III–III	0–0–0	IV–II–0–III	I–0–0–I
F. moniliforme erumpens II		III–III	0–I–0	V–V–IV	I–II–II–I
F. moniliforme minus		III–III	0–0–I–0	V–V	0–0–V–V
F. moniliforme subglutinans		III–III	0–I–0–0	IV–V–III–III	I–0–IV–0
F. neoceras	I–I	0–0	0–0–0–0	III–0–III–II	0–0–I–I
F. niveum 1		II–III	0–0–0–0	V–V–V	III–III–III
F. niveum 2		III–IV	0–0–0–0	V–V–V	III–II–III–V
F. orthoceras		III–IV	0–0–0–0	V–V–V	0–0–V–V
F. orthoceras triseptatum		V–V	II–II–0–0	IV–V–V–V	0–0–V–III
F. ossicolum 1	V–V	0–0–0	0–0–0–0	II–II–III	0–0–III–0
F. ossicolum 2	III–III	0–0	0–0–0–0	V–IV–III–0	0–0–0–0
F. oxysporum 1		III–III	I–I–0–0	V–V–V–I	I–0–0–II–V
F. oxysporum 2		IV–IV	0–I–I–I	V–V–II–V	0–0–I–V
F. oxysporum nicotianae		IV–IV	II–II–II–II	V–V–V–V	0–0–0–0–IV
F. paspalicola	0–IV–0–0	0–0	0–0–0	IV–0–IV–IV	0–0–0–0

TABLE 10.—(*Continued*)

Organism	Tribromphenol sodium salt			Sodium 2.4 dinitrophenoxide	
	0.001%	0.0025%	0.005%	0.01%	0.02%
F. pirinum	V–V	0–0	0–0–0	V–II–II–II	0–0–0–0
F. poae I		V–V	0–I–0–I	V–V–III–III	I–II–II–I
F. poae II		II–II	0–0–0–0	V–V–0–0	0–I–0–I
F. polymorphum	V–V	0–0	0–0–0–0	IV–I–II–II	0–0–V–V
F. pusillum		V–V	I–I–I–I	V–V–V–V	V–0–0–V
F. radicicola		V–V	II–I–II	V–V–IV	II–IV–V–IV
F. rhizochromatistes		IV–V	II–0–0–II	V–V–V–V	II–0–0–I
F. rhizochromatistes microsclerotium		III–V	II–0–0–II	V–V–V–V	0–0–0–0
F. sambucinum 1		II–III	0–0–0–0	IV–V–V–V	V–I–I–V
F. sambucinum 2	V–V	0–0	0–0–0–0	II–II–I–V	0–0–0–0
F. scirpi	III–III	0–0	0–0–0	0–0–0–II	III–II–0–0
F. sclerostromaton		III–III	II–I–0–0	V–V–V–0	I–I–I–I
F. semitectum 1–I	III–III	0–0–0	0–0–0	III–V–IV	III–II–0–III
F. semitectum 1–II	0–III–0–0	0–0–0	0–0–0	V–V–III–0	0–0–0–II–0
F. semitectum 1–III	III–IV	III–III–III	0–0–0	III–III–III–III	IV–III–0–III–IV
F. semitectum 1–IV	0–0–0–0	0–II–0	0–0–0	V–III–II–II	0–0–0–I–0
F. semitectum 1–V	0–0–0–0	0–0–0	0–0–0	IV–0–0–0	0–0–0–0–III
F. semitectum 1–VI	0–0–0–0	0–0–0	0–0–0	V–V–0–II	0–0–0–II–II
F. semitectum 1–VII	0–II–0–0	0–0–0	0–0–0	IV–0–IV–III	0–0–0–II–III
F. semitectum 1–VIII	IV–V	III–IV–V	I–I–I–I	V–V–V–III	V–0–0–III
F. semitectum 1–IX	0–0–0–0	0–0–0	0–0–0–0	III–III–IV–IV	0–0–0–0–IV
F. semitectum 1–X	V–V	0–I–I	0–0–0	III–0–III	0–0–0–0–II
F. semitectum 1–XI	II–II	0–0–0	0–0–0	V–II–III–V	0–0–0–II–III
F. semitectum 1–XII	0–0–0–0	0–0–0	0–0–0–0	III–IV–III–II	0–0–0–II–0
F. semitectum 1–XIII	0–II–0–0	0–0–0	0–0–0	V–V–V–0	0–0–0–V–0
F. semitectum 1–XIV	0–0–0–0	0–0–0	0–0–0–0	III–III–II–III	0–0–0–I–0
F. semitectum 1–XV	0–0–0–0	0–0–0	0–0–0	0–III–0–0	0–0–0–0–0
F. semitectum 1–XVI	0–0–0–0	0–0–0	0–0–0	IV–IV–0–0	0–0–0–II–0
F. semitectum 1–XVII	IV–V	I–I–I	0–0–0–0	III–V–IV–III	0–0–0–II–III
F. semitectum 1–XVIII	V–V	I–0–I	0–0–0–0	0–0–III–II	0–0–0–0–0
F. semitectum 2		I–II	0–0–0–0	III–III–IV–III	IV–0–0–III–IV
F. solani 1		V–V	II–0–0–0	V–V–V	V–V–V
F. solani 2–I		V–V	I–II–II–II	V–V–V–V	0–0–0–III

TABLE 10.—(*Continued*)

Organism	Tribromphenol sodium salt			Sodium 2.4 dinitrophenoxide	
	0.001%	0.0025%	0.005%	0.01%	0.02%
F. solani 2–II		V–V	II–II–II–II	IV–IV–IV–V	IV–V–IV–IV
F. solani 3–I		V–V	I–I–I–I	V–V–V–V	V–V–II–V
F. solani 3–II		II–II	0–I–I–I	V–V–V	I–II–III–I
F. solani 3–III		II–III	0–I–I–I	V–V–V–V	V–II–II–II
F. solani 4–I	V–V	0–0	0–0–0	V–V–V	0–0–I–I
F. solani 4–II		V–V	0–0–0	V–V–V	V–V–III–III
F. solani minus 1–I		III–III	II–II–II–II	V–V–V	V–II–I–II
F. solani minus 1–II		II–III	0–0–I–0	V–V–III	I–I–II–II
F. solani minus 2–I		V–V	I–III–II–IV	V–V–V–V	V–V–II–V
F. solani minus 2–II		II–III	0–0–I–0	V–V–V	II–II–II–V
F. solani suffuscum		II–II	0–0–0	V–III–V	II–II–II–III
F. spinaceae 1		V–V	II–0–I–II	V–V–V	III–III–III–V
F. spinaceae 2		III–III	0–I–I–II	V–V–V	V–V–IV–V
F. striatum 1–I		V–V	I–II–II–II	V–V–V	I–II–0–III
F. striatum 1–II		V–V	I–I–I–I	V–V–V	I–I–IV–I
F. striatum 2		V–V	II–II–II–II	V–V–V	IV–IV–V–V
F. striatum 3–I		III–III	0–II–0–I	V–V–V	II–I–III–V
F. striatum 3–II		IV–IV	0–II–0	V–V–V	II–II–I–III
F. sulphureum 1	V–V	I–I–III	0–0–0–0	V–0–V–V	0–0–0–0
F. sulphureum 2	III–III	0–0	0–0–0–0	III–III–IV–III	0–0–0–0
F. sulphureum 3		II–II	0–0–0–0	III–IV–0–III	0–0–II–0–0
F. theobromæ		II–II	0–I–0–0	V–V–V–V	0–0–II–II
F. tracheiphilum		III–III	I–0–I–0	V–III–V–V	II–0–0–I
F. trichothecioides 1		IV–IV	0–0–0	V–V–V–V	0–I–II–0
F. trichothecioides 2–I	I–0	0–0	0–0–0	IV–IV–0–0	V–0–0–0–0
F. trichothecioides 2–II		II–I	0–0–0–0	III–0–III	0–0–0–0
F. trichothecioides 2–III	II–III	0–0	0–0–0	I–III–0–0–IV	0–0–0–0
F. trichothecioides 3–I	V–V	0–0	0–0–0	V–V–V–V	0–0–II–V
F. trichothecioides 3–II	V–V	0–0	0–0–0	0–0–V–V	0–0–0–0
F. trichothecioides 4–I	0–0–0–0	V–V–0–0	0–0–0–0	IV–V–III–V	0–0–0–0
F. trichothecioides 4–II		IV–IV	0–0–0–0	V–V–V–V	V–0–0–0
F. trichothecioides 5		III–III	0–0–0–0	II–V–V–II	0–0–III–III
F. trichothecioides 6		II–II	0–0–0	V–V–V	0–0–V–I
F. trichothecioides 7–I		II–II	0–0–0–0	V–V–V	0–0–0–I

TABLE 10.—(*Continued*)

Organism	Tribromphenol sodium salt			Sodium 2.4 dinitrophenoxide	
	0.001%	0.0025%	0.005%	0.01%	0.02%
F. trichothecioides 7–II	II–II	0–0	0–0–0–0	0–0–V–0	0–0–0–II
F. tricinctum 1–II	III–IV	0–0–0	0–0–0	II–II–II–I	I–I–0–I
F. tricinctum 1–III	III–III	II–III–0	0–0–0	0–0–II	0–0–0–0
F. tricinctum 1–IV	IV–IV	II–III–0	0–0–0	II–0–II–II	I–I–0–I
F. tricinctum 1–V	II–III	I–I–0	0–0–0	II–II–I–I	I–II–I
F. tricinctum 1–VI	III–IV	I–I–0	0–0–0	I–I–0–II	0–0–0–0
F. tricinctum 1–VII	II–III	0–0–0	0–0–0	0–II–I	I–I–I–I
F. tricinctum 1–VIII	III–I	I–0–0	0–0–0	0–0–II–0	0–0–0–0
F. tricinctum 1–IX	IV–III	I–II–0	0–0–0	II–III–I–II	0–0–0–0
F. tricinctum 2	III–III	0–I	0–0–I–0	II–0–V–II	0–0–I–0
F. uncinatum		II–II	0–0–0	III–IV–III–IV	0–0–I–I
F. urticearum I	0–I–0–0	0–0	0–0–0	I–0–II–0	0–0–0–0
F. urticearum II	I–0–0–0	0–0	0–0–0	II–I–0–0	0–0–I–0
F. vasinfectum		I–III	0–0–0–0	V–V–II–V	I–II–III–I
F. viride 1		V–V	I–II–II–II	V–III–IV	V–V–I–IV
F. viride 2		I–II	0–0–0–0	V–V–III–V	0–II–I–IV
F. viticola		I–II	0–0–0–I	III–III–III–III	0–0–III–III
F. zonatum 1		II–II	I–0–0–0	V–V–IV–III	I–0–0–II
F. zonatum 2		III–III	I–0–0–I	III–III–V–III	I–II–0–I–II
F. zonatum 3		III–III	I–II–II–II	III–III–III–III	III–III–II–II

GROWTH IN PRESENCE OF IODIC ACID AND PHENYL ACETIC ACID

Two concentrations of iodic acid (0.05 and 0.075 per cent) and one concentration of phenyl acetic acid (0.03 per cent) have been used in this experiment. Fusaria exhibit a very narrow margin of toleration to these substances, especially to phenyl acetic acid. 0.1 per cent of iodic acid inhibited the growth in nearly every one of the strains tested. 0.05 per cent of phenyl acetic acid had a similar action, but when its concentration was lowered to 0.02 per cent, such a large number of organisms made a vigorous growth that it was not found worthwhile to include the results in Table 11.

It is not necessary to make a detailed analysis of table 11, because it illustrates, although in a somewhat different way, the same principle revealed by all the foregoing tables, namely, that individual strains may vary greatly within the sphere of the same species, that each individual may vary within its own life period, that fluctuation is the most constant characteristic of these organisms, and that attempts at standardization may never be very fruitful.

The responses towards iodic acid were tested further in case of the more erratic strains, and their reactions, more conflicting than ever, are given below:

DISCUSSION

The foregoing tables show that the extreme variability and inconstancy which exist within the strains of the same species, and within the successive reactions of the same organism, preclude the possibility of developing a simple scheme of classification. We have seen that rate of growth, color of substratum and mycelium, type of fruiting, presence and absence of different kinds of spores, temperature relations, and reactions towards acids, dyes, and toxic substances are not constant. To the superficial observer this may appear as a hopeless situation, but a closer analysis of the experimental data reveals that a characteristic line usually maintains its course throughout such inconsistencies and unorthodox behaviors.

Let us consider, for example, the five strains of *F. martii phaseoli*. Strains 1–I, 1–III, and 2–I form pigments, 1–II and 2–II do not; strains 1–I and 2–I produce an abundance of sporodochia, the remainder do not; strains 1–I, 1–III, 2–I, and 2–II form no microconidia, while 1–II does. Strain 1–III is the only one that remains sterile on malt extract agar, and 1–I and 2–I are the only ones capable of forming macroconidia. Strains 1–II and 2–I tolerate 0.25 per cent tannic acid, the remaining three do not. Strains 1–III and 2–II cannot grow in the presence of 0.001 per cent sodium tribromphenate. Strains 2–I and 2–II fail to grow in the presence of acid phenyl acetic. Strain 2–I is the only one that can grow at a temperature of 5° C. On the other hand, all five strains are slow growers, all are extremely

TABLE 11.—*Growth in presence of iodic acid and Phenyl acetic acid (2 weeks at 25° C.)*

Organism	Iodic Acid		Acid Phenyl Acetic
	0.05%	0.075%	0.03%
F. acuminatum 1	III–III–IV–V	0–0–0	V–II–0–0–III
F. acuminatum 2–I	V–IV–III–III	0–0–V	0–0–0–0
F. acuminatum 2–II	V–V–V–V	0–0–V	0–0–0–0
F. acuminatum 2–III	V–V–0–0	0–0–IV	0–0–0–0
F. acuminatum 2–IV	V–V–V–0	0–0–0	0–0–0–III–V
F. acuminatum 2–V	III–V–IV–III	II–II–0	0–0–0–III
F. acuminatum 2–VI	V–IV–V–0	0–0–0	0–0–0–0
F. acuminatum 2–VII	V–V–V–0	0–0–0	0–0–0–0
F. acuminatum 2–VIII	IV–V–0–0	0–0–0	0–0–0–0
F. alluviale	II–II–I–0	0–0–0	V–0–0–V–V
F. anthophilum	III–V–I–0	0–0–0	0–0–0–I–II
F. aquaeductuum pusilum	III–I–III–II	0–0–0	0–0–0–II–II
F. aquaeductuum volutum	I–0–I–0	0–I–0	0–0–0–II–II
F. arcuatum	V–V–IV	0–0–IV	0–0–0–III–V
F. asclerotium	V–IV–0	IV–0–V	V–V–III–IV
F. aurantiacum	IV–V–V–IV	0–0–V	V–V–V–V
F. batatatis 1	0–0–I	0–0–0	V–V–V–V
F. batatatis 2	IV–V–V–V	0–0–V	V–IV–III–V
F. bulbigenum	IV–V–V–V	0–V–V	V–V–V–V
F. bullatum I	IV–IV–V–V	0–0–V	0–0–0–0–V
F. bullatum II	V–IV–IV–V	0–IV–V	V–V–0–IV–IV
F. bullatum brevius	V–V–V–V	0–IV–IV	V–V–V–V
F. camptoceras I	IV–IV–IV–IV	0–0–IV	0–0–0–IV–V
F. camptoceras II	IV–IV–III–IV	IV–0–0	0–0–0–IV–IV
F. caudatum	IV–IV–0–0–0	0–0–V	V–IV–0–0–0
F. chlamydosporum I	V–V–V–0	0–0–IV	I–0–0–0–0
F. chlamydosporum II	0–III–III–III	0–0–0	II–II–0–0–II
F. chlamydosporum III	V–V–III–V	0–0–IV	0–0–0–0
F. chenopodinum	III–IV–0–IV	0–0–0	0–0–0–III–0
F. coeruleum 1–I	I–0–II–I	0–0–0	IV–IV–IV–V
F. coeruleum 1–II	0–0–0–0	0–0–0	V–V–IV–V
F. coeruleum 2	II–II–V–0	0–0–0	V–V–V–III
F. coeruleum 3	0–0–V–0	0–0–0	0–0–0–0
F. commutatum	II–I–I–0	0–0–0	0–0–0–0
F. conglutinans	V–V–V–0	V–0–0	V–V–IV–V
F. cubense 1	V–V–V–V	0–V–V	V–V–0–V
F. cubense 2	V–V–0–V	V–0–V	V–III–III–V
F. culmorum 1–I	V–0–V–0	0–0–0	0–0–0–0
F. culmorum 1–II	0–0–V	0–0–IV	0–0–0–0
F. culmorum 2	V–V–0–0	V–0–0	0–0–0–0
F. culmorum 3–I	0–V–0–0	I–IV–V	0–0–V–V
F. culmorum 3–II	V–IV–0–0	0–0–0	0–0–II–III
F. culmorum 4–I	V–0–V–V	0–0–III	V–0–0–I
F. culmorum 4–II	IV–IV–III–0	0–0–V	II–I–0–0–0
F. culmorum 5	V–V–V–0	0–0–I	IV–IV–V–0
F. culmorum 6–I	V–V–V–0	V–V–V	V–V–V–V
F. culmorum 6–II	V–V–III–II	V–0–0	V–V–V–V
F. decemcellulare	V–V–0–0–0–0	0–0–0	0–0–0–III–V
F. dimerum	V–V–V–0	V–0–0	V–I–0–0–0
F. diversisporum	V–V–V–0	V–0–0	V–V–IV–V
F. ensiforme	0–0–0–0	0–0–0	0–0–0–0
F. eumartii 1–I	0–0–0–0	0–0–0	0–0–II–0–0
F. eumartii 1–II	I–0–0–I	0–0–0	V–V–V–V
F. eumartii 2–I	III–I–0	0–0–0	0–0–II–V
F. eumartii 2–II	0–V–I–V	0–0–0	II–I–0–0–0
F. eumartii 3	0–V–I–0	0–0–0	0–0–0–II–III

TABLE 11.—(*Continued*)

Organism	Iodic Acid		Acid Phenyl Acetic
	0.05%	0.075%	0.03%
F. euoxysporum	V–0–V–V	0–0–V	V–V–IV–V
F. falcatum	V–V–V–0	V–0–0	0–0–0–0
F. filiferum I	IV–V–IV–V	0–0–III	V–IV–0–0–V
F. filiferum II	IV–IV–0	V–V–IV	0–0–V–0–0
F. flocciferum	V–0–0–0	0–0–0	V–V–V–V
F. fructigenum	III–V–V–0	0–0–0	IV–IV–V–0–0
F. fructigenum maius 1	V–V–V–V	V–V–V	IV–IV–V–V
F. fructigenum maius 2–I	V–V–V–IV	0–0–0	V–V–0–V
F. fructigenum maius 2–II	IV–V–V	0–V–0	IV–IV–IV–V
F. fructigenum maius 2–III	V–V–V–0	0–0–0	V–V–V–V
F. fructigenum maius 2–IV	V–V–V–0	0–0–0	V–V–IV–V
F. fructigenum maius 2–V	V–IV–0	0–III–I	V–V–III–V
F. fructigenum maius 2–VII	0–0–0–0	0–0–0	V–V–0–V
F. fructigenum maius 2–VIII	V–0–V–0	0–0–0	V–III–V–V
F. fructigenum maius 3–I	V–V–V	0–0–0	0–0–0–V–V
F. fructigenum maius 3–II	V–II–V	0–0–0	0–0–0–V–V
F. fructigenum maius 3–III	V–0–V–V	0–0–I	0–0–0–IV–V
F. gibbosum	IV–V–V–V	0–0–V	IV–V–0–III
F. graminum 1	0–0–V–0	0–I–V	0–0–0–0–V
F. graminum 2–I	III–V–V–0	V–V–0	0–0–0–II–V
F. graminum 2–II	V–V–V–0	0–0–III	0–0–0–V–III
F. herbarum 1	III–III–IV–III	0–II–0	II–0–0–V
F. herbarum 2	V–V–IV–V	0–III–0	III–II–V–V
F. herbarum pirinum I	IV–V–V–0	0–II–0	V–V–V–V
F. herbarum pirinum II	II–IV–V–0	0–0–0	III–III–III–V
F. herbarum gibberalloides I	IV–V–V–IV	0–0–0	V–V–0–III
F. herbarum gibberalloides II	V–IV–V	0–0–0	III–III–IV–IV
F. hyperoxysporum 1	V–V–V	0–0–V	V–V–V–V
F. hyperoxysporum 2	V–V–V	IV–IV–V	IV–IV–IV–IV
F. incarnatum I	V–V–IV–V	V–IV–IV	V–V–IV–IV
F. incarnatum II	III–IV–IV–III	III–0–0	V–V–0–V
F. incarnatum IV	III–IV–I–II	0–III–V	0–0–III–IV
F. incarnatum V	III–III–0–III	IV–II–IV	IV–IV–0–0
F. javanicum 1	II–0–0–0	0–0–0	V–V–V–V
F. javanicum 2	V–V–V–0–0	V–V–0	II–0–0–V–V
F. lateritium I	V–IV–0–0	0–0–0	0–0–II–III
F. lateritium II	V–0–V–0	0–0–I	0–0–0–II–V
F. lini	I–I–0	0–0–0	V–V–V–V
F. lolii	I–0–0	0–0–0	0–0–0–0–0
F. loncheceras	V–V–V–V	0–0–V	V–V–IV–V
F. loncheceras microsporum	V–V–V–0	0–0–V	V–V–IV–IV
F. longipes	V–V–V–0	II–0–0	0–0–0–0
F. lutulatum I	IV–V–IV–IV	IV–IV–IV	III–III–0–0–0
F. lutulatum II	III–V–IV–IV	IV–0–0	IV–IV–IV–III
F. lycopersici 1	V–V–0–0	0–0–IV	V–V–V–V
F. lycopersici 2	V–V–V–0	V–0–V	V–V–V–V
F. malli	V–V–V–0–0	V–V–V	IV–V–IV–IV
F. macroceras	III–0–0–0	0–0–0	0–0–0–0
F. martii 1	0–0–0	0–0–0	V–V–V–V
F. martii 2	V–V–V–V	V–V–V	V–V–V–V
F. martii 3–I	0–0–0–I	0–0–0	III–III–II–V
F. martii 3–II	0–0–0–0	0–0–0	V–V–III–III
F. martii minus	II–II–II–0	0–0–0	V–V–V–V
F. martii phaseoli 1–I	I–0–II–I	0–0–0	IV–I–0–0–0
F. martii phaseoli 1–II	0–0–0–0	0–0–0	V–V–III–II–V
F. martii phaseoli 1–III	0–0–0–0	0–0–0	V–V–0–II–II

TABLE 11.—(*Continued*)

Organism	Iodic Acid		Acid Phenyl Acetic
	0.05%	0.075%	0.03%
F. martii phaseoli 2–I	0–0–0	0–0–0	0–0–0–0
F. martii phaseoli 2–II	0–0–V–0	0–0–0	0–0–0–0
F. martii pisi	V–V–V–V	0–V–V	IV–IV–IV–IV
F. martii viride	0–0–0–V	0–0–0	V–V–V–V
F. moniliforme 1	V–V–V–IV	V–IV–IV	IV–IV–IV–IV
F. moniliforme 2	V–V–V–0	0–V–V	V–V–V–V
F. moniliforme 3	V–V–V–V	0–0–0	V–IV–IV–II
F. moniliforme 4	V–0–V–V	0–V–V	V–V–V–V
F. moniliforme 5	V–V–V–V	V–0–V	IV–IV–II–V
F. moniliforme erumpens I	V–V–V	0–0–0	IV–IV–IV–IV
F. moniliforme erumpens II	V–0–IV	V–0–0	V–V–V–V
F. moniliforme minus	V–V–V	IV–IV–V	V–V–V–V
F. moniliforme subglutinans	V–V–V–0	0–0–0	V–V–V–V
F. neoceras	V–V–V–V	0–0–0	V–V–IV–V
F. niveum 1	V–V–IV–IV	V–0–0	III–III–0–III
F. niveum 2	V–0–0–0	V–0–0	V–V–V–V
F. orthoceras	V–V–V–0–V	0–0–V	V–V–V–V
F. orthoceras triseptatum	V–IV–V–0–IV	0–V–V	V–V–0–V
F. ossicolum 1	IV–IV–III	IV–IV–IV	0–0–0–IV–IV
F. ossicolum 2	V–IV–V–0	0–V–0	0–0–0–0–0
F. oxysporum 1	V–V–V	0–V–V	V–V–V–V
F. oxysporum 2	V–V–V	0–0–0	V–V–V–V
F. oxysporum nicotianae	V–V–V	0–V–V	V–IV–IV–IV
F. paspalicola	0–0–II–0	0–0–0	0–0–0–0
F. pirinum	V–V–V–0	0–0–0	0–0–III–0
F. poae I	V–V–0–0–V	0–0–0	V–V–V–V
F. poae II	V–V–V–V	V–0–0	V–V–V–V
F. polymorphum	IV–I–V–0	0–0–0	II–II–IV–V
F. pusillum	0–0–V–0	0–0–0	V–III–0–V
F. radicicola	III–0–0	0–0–0	V–V–V–V
F. rhizochromatistes	V–V–V–V	V–0–V	V–IV–IV–V
F. rhizochromatistes microsclerotium	IV–V–V–0–0	0–0–V	0–V–IV–V
F. sambucinum 1	III–III–III–III	I–I–I	IV–IV–V–V
F. sambucinum 2	V–V–V–0	0–0–0	V–IV–III–I–0
F. scirpi	IV–IV–IV–0	0–II–V	III–III–0–II
F. sclerostromaton	V–V–0–V	0–0–0	V–IV–IV–V
F. semitectum 1–I	III–I–III–III	III–IV–V	V–V–0–0–0
F. semitectum 1–II	IV–V–IV–V	0–0–0	II–0–IV–0–0
F. semitectum 1–III	III–III–III–III	0–IV–III	IV–IV–IV–IV
F. semitectum 1–IV	V–III–III–IV	0–0–0	0–0–0–0
F. semitectum 1–V	V–V–0–0	0–0–0	0–0–0–III–V
F. semitectum 1–VI	V–0–V–0	0–0–0	0–0–0–0–0
F. semitectum 1–VII	IV–IV–III–V	0–II–0	IV–V–0–IV
F. semitectum 1–VIII	V–V–V–V	V–V–V	IV–IV–IV–IV
F. semitectum 1–IX	III–III–IV–IV	III–III–0	IV–IV–III–III
F. semitectum 1–X	V–V–0	V–IV–IV	V–0–0–0–V
F. semitectum 1–XI	V–V–IV–0	0–0–0	0–0–0–III–III
F. semitectum 1–XII	III–IV–III–V	0–0–0	0–0–0–V–V
F. semitectum 1–XIII	IV–0–V–V	0–0–0	IV–0–V–0
F. semitectum 1–XIV	III–IV–III–0	0–0–0	0–0–III–IV
F. semitectum 1–XV	III–III–V–0	0–0–0	0–0–0–0
F. semitectum 1–XVI	IV–IV–V–0	0–0–0	V–0–0–0–0
F. semitectum 1–XVII	V–IV–V–IV	0–0–V	IV–IV–III–V
F. semitectum 1–XVIII	V–IV–V–III	IV–IV–IV	V–IV–0–0–0
F. semitectum 2	III–III–III–III	IV–IV–IV	IV–IV–III–III

TABLE 11.—(*Continued*)

Organism	Iodic Acid		Acid Phenyl Acetic
	0.05%	0.075%	0.03%
F. solani 1	I–0–I–0	0–0–0	V–V–V–V
F. solani 2–I	V–V–V–0	V–V–V	V–V–V–V
F. solani 2–II	I–I–I–I	0–0–0	V–V–III–V
F. solani 3–I	0–IV–0–III	0–0–0	V–V–V–V
F. solani 3–II	0–0–I	0–0–0	V–V–V–IV–III
F. solani 3–III	V–V–III	0–0–0	V–V–V–IV–V
F. solani 4–I	III–II–II	0–0–0	IV–V–III–V
F. solani 4–II	III–III–V–0	0–0–0	III–III–III–V
F. solani minus 1–I	0–V–0–0	0–0–0	II–II–V–V
F. solani minus 1–II	I–I–0–0	0–0–0	V–V–III–V
F. solani minus 2–I	II–0–0	0–0–0	V–V–V–V
F. solani minus 2–II	0–0–0	0–0–0	III–IV–V–V
F. solani suffuscum	V–III–I–0	0–0–0	V–IV–IV–V
F. spinaceae 1	V–V–V–0	0–0–V	IV–V–IV–V
F. spinaceae 2	V–V–V–V	V–0–0	V–V–V–V
F. striatum 1–I	II–I–I–0	0–0–0	II–III–V–V
F. striatum 1–II	0–0–0–0	0–0–0	III–II–V–V
F. striatum 2	0–0–0–0	0–0–0	V–V–IV–V
F. striatum 3–I	I–0–0–0	0–0–0	V–V–V–V
F. striatum 3–II	0–0–I	0–0–0	V–V–V–V
F. sulphureum 1	V–0–V–V	0–0–0	IV–IV–0–0–0
F. sulphureum 2	III–III–IV–III	0–0–III	0–0–0–II–III
F. sulphureum 3	IV–IV–IV–IV	0–0–0	0–0–IV–IV
F. theobrome	0–0–0–0	0–0–0	I–II–V–III–IV
F. tracheiphilum	V–V–V–0	V–V–V	V–II–III–V
F. trichothecioides 1	III–V–IV–V	0–0–0	IV–IV–IV–V
F. trichothecioides 2–I	I–0–V–0	V–0–0	IV–0–0–0–0
F. trichothecioides 2–II	III–IV–0	0–0–0	V–V–0–0–IV
F. trichothecioides 2–III	IV–V–V–V	V–0–0	0–0–0–0–0
F. trichothecioides 3–I	V–V–V–V	V–0–0	0–0–0–0
F. trichothecioides 3–II	V–V–V–0	0–0–V	0–0–0–0
F. trichothecioides 4–I	0–0–0	0–0–0	0–0–0–0
F. trichothecioides 4–II	IV–V–IV	0–0–0	III–0–0–0–0
F. trichothecioides 5	V–V–0–0–0	0–0–V	V–V–III–V
F. trichothecioides 6	V–V–V–V	0–0–V	V–V–V–V
F. trichothecioides 7–I	0–I–0–0	0–0–0	III–II–I–V
F. trichothecioides 7–II	0–V–V–0	0–0–0	0–0–V–0
F. tricinctum 1–III	III–0–III	0–0–0	V–III–0–II
F. tricinctum 1–IV	III–0–0–0	I–0–0	III–0–0–III
F. tricinctum 1–V	V–V–0–0	0–0–0	V–V–II–V
F. tricinctum 1–VI	III–V–0–V–0	0–0–0	0–0–III–IV
F. tricinctum 1–VII	0–V–0	0–0–0	II–0–0–IV
F. tricinctum 1–VIII	0–0–0–0	0–0–0	II–0–0–III
F. tricinctum 1–IX	III–0–0–0	III–0–0	0–0–0–III–IV
F. tricinctum 2	III–III–IV–III	0–0–0	III–II–II–IV
F. uncinatum	IV–V–V–0	0–0–0	III–III–II–III
F. urticearum I	0–IV–V–0	0–0–0	0–0–0–0
F. urticearum II	V–III–0–0	0–0–0	0–0–0–II
F. vasinfectum	V–V–V–V	0–0–0	V–IV–IV–V
F. viride 1	0–0–0–0	0–0–0	V–V–V–V
F. viride 2	V–0–V–0	I–V–V	V–V–0–IV
F. viticola	III–I–III–0	I–0–0	IV–IV–III–III
F. zonatum 1	IV–V–V–0	0–0–0	V–V–III–IV
F. zonatum 2	IV –V–V–V	V–V–V	IV–IV–IV–III–IV
F. zonatum 3	V–V–V–0	V–0–0	IV–III–III–IV

F. asclerotium → 0.05 per cent iodic acid = V IV 0 → nutrient solution = 0

F. alluviale → 0.05 per cent iodic acid = II II I 0 → nutrient solution = 0

F. anthophylum → 0.05 per cent iodic acid = III V I 0 → nutrient solution = 0

F. coeruleum 2 → 0.025 per cent iodic acid = II II V 0 → nutrient solution = V → agar → 0.05 per cent iodic acid = III II I

F. coerulem 3 → 0.05 per cent iodic acid = 0 0 V 0 → nutrient solution → = 0

F. culmorum I → 0.05 per cent iodic acid = V 0 V 0 → nutrient solution = 0

F. conglutinans → 0.05 per cent iodic acid = V V V 0 → nutrient solution = 0

F. chlamydosporum I → 0.05 per cent iodic acid = V V V 0 → nutrient solution = 0

F. commutatum → 0.05 per cent iodic acid = II I I 0 → nutrient solution = V → agar → 0.05 per cent iodic acid = 0 0 0

dimerum → 0.05 per cent iodic acid = V
V
V
0 → nutrient solution = 0

diversisporum → 0.05 per cent iodic acid = V
V
V
0 → nutrient solution = 0

decemcellulare → 0.05 per cent iodic acid = V
V
0
0 → nutrient solution = V → agar → 0.05 per cent iodic acid = 0
0
0

eumartii 2–I → 0.05 per cent iodic acid = III
I
0 → nutrient solution = 0

eumartii 2–II → 0.05 per cent iodic acid = 0
V
I
V → agar → 0.05 per cent = 0
0 → nutrient solution = 0
V → agar → 0.05 per cent idodic acid = 0
0
0

falcatum → 0.05 per cent iodic acid = V
V
V
0 → nutrient solution = 0

filiferum II → 0.05 per cent iodic acid = IV
IV
0 → nutrient solution = 0

flocciferum → 0.05 per cent iodic acid = V
0
0
0 → nutrient solution = 0

fructigenum → 0.05 per cent iodic acid = III
V
V
0 → nutrient solution = 0

F. fructigenum maius 2–III →0.05 per cent iodic acid = V, V, V
0 → nutrient solution = 0

F. fructigenum maius 2–IV → 0.05 per cent iodic acid = V, V, V
0 → nutrient solution = 0

F. fructigenum maius 2–V → 0.05 per cent iodic acid = V, IV
0 → nutrient solution = 0

F. fructigenum maius 2–VIII → 0.05 per cent iodic acid = V, 0, V
0 → nutrient solution = 0

. gibbosum → 0.05 per cent iodic acid = IV, V, V
V → agar → 0.05 per cent = 0
0 → nutrient solution = 0

V → agar → 0.05 per cent iodic acid = 0, 0, III

. javanicum 2 → 0.05 per cent iodic acid = V, V, 0
0 → nutrient solution = 0

lolii → 0.05 per cent iodic acid = I, 0
0 → nutrient solution = 0

longipes → 0.05 per cent iodic acid = V, V, V
0 → nutrient solution = 0

lycopersici 1 → 0.05 per cent iodic acid = V, V, 0
0 → nutrient solution = 0

macroceras → 0.05 per cent iodic acid = III / 0 / 0 → nutrient solution = 0

malli → 0.05 per cent iodic acid = V / V / 0 → nutrient solution = 0

martii phaseoli 2–II → 0.05 per cent iodic acid = 0 / 0 → nutrient solution = 0

martii viride → 0.05 per cent iodic acid = 0 / 0 / 0 → V → agar → 0.05 per cent iodic acid = 0 / 0 → nutrient solution = V → agar → 0.05 per cent iodic acid = 0 / 0

moniliforme 2 → 0.05 per cent iodic acid = V / V / V → 0 → nutrient solution = 0

moniliforme erumpens II → 0.05 per cent iodic acid = V / 0 / IV → nutrient solution = 0

moniliforme subglutinans → 0.05 per cent iodic acid = V / V / V → 0 → nutrient solution = 0

orthoceras → 0.05 per cent iodic acid = V / V / 0 → V → agar → 0.05 per cent iodic acid = V / V / V

ossicolum 2 → 0.05 per cent iodic acid = V / IV / V → 0 → nutrient solution = 0

F. polymorphum → 0.05 per cent iodic acid = IV / I / V
0 → nutrient solution = 0

F. pusilum → 0.05 per cent iodic acid = 0 / 0 / V
0 → nutrient solution = V → agar → 0.05 per cent iodic acid = 0 / 0 / 0

F. rhizochromatistes microsclerotium → 0.05 per cent iodic acid = IV / V / V / 0
0 → nutrient solution = 0

F. semitectum 1–V → 0.05 per cent iodic acid = V / V / 0
0 → nutrient solution = 0

F. semitectum 1–X → 0.05 per cent iodic acid = V / V
0 → nutrient solution = 0

F. semitectum 1–XIII → 0.05 per cent iodic acid = V → agar → 0.05 per cent iodic acid = 0 / 0 / 0 / 0
0 → nutrient solution = 0

F. scirpi → 0.05 per cent iodic acid = IV / IV / IV
0 → nutrient solution = 0

F. sclerostromaton → 0.05 per cent iodic acid = V / V / 0 / V → agar → 0.05 per cent iodic acid = V / V / V

Fusarium solani 4–II → 0.05 per cent iodic acid = III / III / V
0 → nutrient solution = V → agar → 0.05 per cent iodic acid = 1 / 1 / 0

F. solani 3–I → 0.05 per cent iodic acid = 0 IV 0 III → agar → 0.05 per cent iodic acid = 0 0 → nutrient solution = V → agar → 0.05 per cent iodic acid = 0 0 0

F. solani minus I → 0.05 per cent iodic acid = 0 V 0 0 → agar → 0.05 per cent iodic acid = 0 0 0

F. solani suffuscum → 0.05 per cent iodic acid = V III I 0 → nutrient solution = 0

F. sambucinum 2 → 0.05 per cent iodic acid = V V V 0 → nutrient solution = 0

F. tricinctum 1–VI → 0.05 per cent iodic acid = V III V 0 → nutrient solution = 0 0

F. trichothecioides 5 → 0.05 per cent iodic acid = V V 0 0 → nutrient solution = 0

F. zonatum 1 → 0.05 per cent iodic acid = IV V V 0 → nutrient solution = 0

sensitive to tartaric acid, all tolerate comparatively high concentrations of malachite green, mercurochrome, and sodium dinitrophenoxide, all are sensitive to iodic acid, and finally all fail to grow at 37.5° C. If we add to these similarities of behavior the known morphological and pathological relationships of these organisms, no one will doubt that, regardless of a number of important dissimilarities, all these strains really belong to the same species. Such is the case with the great majority if not all of the strains used in this work.

The present scheme of classification of Fusaria is very largely based on the morphology of the spores, especially that of the macroconidia. It must therefore follow that before an identification can be made, macroconidia must be produced. Here again the worker may sometimes find himself confronted by all sorts of difficulties. A glance at table 8 will show that there is a marked variation between the strains of the same species in their ability to form the three types of spores. It may be argued that only one kind of nutrient agar can hardly be expected to bring out all of the spores in all of the strains. Very true; it is even quite permissible to extend this statement and say that not even half a dozen or a dozen different agars can promote perfect sporulation in all strains. There always will be the same baffling contrast of sterility, partial sterility, and abundant sporulation exhibited within the limits of the same species. What is the investigator to do if a species of Fusarium constantly fails to produce macroconidia? Even if he finally does find a medium on which all three or any one of the desired spores will be produced, how will it be possible to make an identification unless the measurements of the corresponding type species are also made from the spores produced on the same medium? If a subculture of the type species is secured, what probability is there that it will sporulate on this agar? And, if it does sporulate, what are the exponents of "natural conditions" going to say about the use of artificial propagation? How is the worker to know that the culture is pure, that it has not mutated, has not "degenerated" by being carried on artificial media? And above all, who is going to make any one strain the ruling force within the specific orbit? Why should not another strain be just as typical and worthy of the honored status of type specimen? Furthermore, what is the value of spore measurements unless all conditions are standardized?

Size and shape factors are extremely flexible and vitally affected by the presence of various chemical substances in their food as shown by Brown and Horne (2). An examination of almost any illustration of Fusarium spores reveals the same thing. The macroconidia of *F. camptoceras,* from 8-day cultures on hard potato agar, as illustrated by Reinking and Wollenweber (8) and those from 15-day-old cultures on hard potato agar, are not at all alike. In *F. anguoides* the differences become still sharper when the

illustrations of the conidia from the mycelium of 1-month-old potato-plug cultures are compared with those of conidia obtained from the pionnotes of 18-day-old oatmeal agar, or 1-month-old rice cultures. In *F. longipes,* even a layman can tell that the illustrations of macroconidia taken from the pionnotes of 3-month-old hard potato-agar cultures have nothing in common with the drawings of macroconidia taken from the sporodochia of 1-month-old Alnus-stem cultures. Such more or less sharp differences can be seen in many illustrations of Fusaria when different substrata are used for food. Such being the case, it is quite a doubtful procedure to depend upon spores which may be produced by the use of different agars. It is improbable that one will ever be able to find a universal medium on which all Fusaria will readily reproduce the desired types of spores; and, yet, without standardized and uniform environmental conditions no morphological work can be so conclusive or convincing as one might desire.

Since differences in composition of food are capable of materially altering the size and the form of spores, it is logical to assume that different hosts with their still more complex chemical and physical makeup cannot fail to affect the morphology of the fungus. Nor are we in a position to state that in nature the constant and fierce competition for food does not complicate matters still further. How do the by-products of secondary invaders, such as fungi, bacteria, or protozoa, affect the composition of the host tissue? Since such by-products differ with different organisms, and since no one can seriously say that secondary invaders are always the same regardless of host or locality, the inevitable conclusion is that controlled laboratory conditions are more conducive to uniform sporulation. But can one be justified in discarding morphological factors just because no universal substratum can be found on which free sporulation of all strains of Fusaria will occur? Not at all.

But here is where the value of physiological tests becomes apparent. If the standard agars at our disposal fail to induce the formation of the desired type of spores, some physiological reaction may prove very useful in the identification of the unknown, and no long, often fruitless, search need be necessary for a medium capable of promoting conidium formation. The morphologists are beginning to realize this and employ certain laboratory tests as reliable criteria in their identification keys.

As fungi are studied more and more intensively, and new varieties and forms are every day being identified, the need for additional means of segregation becomes gradually more acute. We may admit that individual variations always will exist, that the intensity of such variations may tend to shape our conception of a true species. We may even go so far as to admit that very often the species may be nothing more than a clumsy device created for our own convenience, that its value may be directly proportional to

the degree of our own ignorance concerning the potentialities of the living protoplasm, yet we cannot get away from the fact that we must put either a true or an arbitrary limitation around some sort of a species sphere, making the species tests sufficiently numerous, conclusive, and flexible to define more clearly any distinct type of organism that we may need to identify. But before such a series of tests can be evolved, a more thorough study of the different strains of the species should be made.

No species is unlimited in its potential possibilities, nor do its variations usually fail to confine themselves to a certain sphere. It becomes the duty of the investigator to approach these boundaries by testing a very large number of individuals. By developing a great number of simple and effective tests, he should be able to construct a scheme whereby the mean of the specific reactions can be reached. After a sufficient amount of data are accumulated, it will not be difficult to select the more comprehensive tests and standards, and discard doubtful or less desirable ones. This sort of work has been thoroughly done in morphology, but in the study of physiological characters, the surface has been barely scratched. The present work is merely a step in this direction and no more. Much additional research is necessary before it can emerge from its formative stage and assume a more directive character. Many additional tests, if developed in the right direction, should relieve the strain that is being put upon morphological methods by the constant appearance of new forms or new variations. If such tests are sufficiently numerous and reliable, the proportion of "misfits" will be reduced to the minimum; for, after all, no organism can vary indefinitely, and its general behavior rarely, if ever, can jump the boundaries of the species.

The underlying causal factors responsible for the variability and inconsistencies of Fusaria may be roughly divided into external and internal groups. The external factors, themselves, are of two types: those which bring about temporary fluctuations without making any kind of impression upon the hereditary qualities of the protoplasm, and second, those that act as stimuli to awaken, stimulate, and perpetuate dissociative potentialities in the fungus. The internal factors are intimately concerned with the biological construction and mechanism of the protoplasm, and seem to bear little or no relationship to the usual environmental conditions. While the appearance of "sectors" in the colonies of Fusaria may not be considered mutations, yet, on the other hand, they can not be classified with the ordinary fluctuations because they appear and perpetuate themselves despite all attempts artificially to control them. It is true that even the most elaborately controlled laboratory conditions are comparatively crude, yet we can not hope to get completely away from our own physical limitations, and must, therefore, draw our conclusions from available data. The environ-

mental factors in the foregoing experiments have been controlled as rigidly as possible, yet the baffling fluctuations and endless inconstancies in the behavior of Fusaria have occurred so frequently and abundantly as to indicate conclusively that such variations cannot be ranked with ordinary fluctuations.

We cannot say that sector formation is due to food differences in a plate of agar because it is very difficult to conceive of such differences and because sectors develop even when the cultures are grown in a liquid medium where the distribution of sugars and salts is homogeneous. Furthermore, any fluctuation caused by food differences is bound to disappear as soon as normal conditions are reestablished. Yet, we find that many of the new characteristics persist for many generations after they have once been separated in pure culture. What is still more significant is the fact that differences arise not only in morphological characters but in vital physiological reactions as well. The same may be said about the possible presence or absence of certain essential elements in the medium. It should be remembered that only one solid agar was used throughout this work; the same quality and quantity of chemicals, the same amount of agar, the same environmental conditions were retained throughout the experiment. Yet even in duplicate and triplicate cultures some glaring inconsistencies continued to appear; one plate would show definite sectors while the others would remain uniformly the same irrespective of the fact that the same stock culture was used in the inoculation work. How can the presence or absence of certain food elements explain the fact that the new type, after being isolated in pure culture and breeding true for a few generations, will again split into a complex colony or revert to its prototype? No other nutrient agar was used, nothing was ever introduced that was not already there, yet reversions continued to occur. Furthermore, the writer has tested many combinations of chemicals with the hope of controlling sector formation, but without any success. Ultraviolet light, hard and soft X-rays and radium were tried with some of the most inconstant types, yet no outstanding effect could be observed. Twelve hours' exposure to the action of radium showed not the slightest change in the growth habit of the colonies. A fluctuating type of *F. moniliforme* was irradiated with X-rays at the rate of 10 and 20 minutes a day for two weeks, yet no change could be observed in its rate of growth, variability, general macroscopic appearance, and the ability to attack corn seedlings. It was also irradiated with ultraviolet light for an hour a day for two weeks, and the only noticeable effect even when a fluorescent dye was added to the medium, consisted of a slower rate of growth and as soon as the irradiation was discontinued the normal rate of growth was renewed.

The effect of the bacteriophage on bacteria, as described by d'Herelle (4) bears a remarkable parallelism with some of the variations recorded in

this paper, especially the reverting fluctuations. The Shiga bacillus, under the influence of bacteriophage, can change into coccus, mucoid, and ultramicroscopic types and become resistant to the attack of the bacteriophage. So long as the ultraparasite is present, the new types of Shiga bacillus persist, and even after the total removal of the bacteriophage they may breed true for a number of generations. Eventually, however, they revert back to the original bacillus type, lose their resistance to drying, to the age factor, and to the phage itself. The struggle between the bacteria and bacteriophage induces the emergence of these new types. It might be suggested that there is a sort of phage operating in Fusaria, but this seems improbable because the writer has observed no trace of lysis, no diminution of vigor, and no other sign except the baffling fluctuations.

All failure artificially to induce colony dissociations notwithstanding, a stimulus must exist in the environmental conditions before a response occurs on the part of the organism. Perhaps a number of factors can and do act as stimuli, but the reason why dissociations do not manifest themselves with unfailing regularity whenever stimuli are applied is that a constant state of flux exists in the protoplasm, and consequently the necessary coordination between the protoplasm and the proper environmental factor cannot be established except at certain intervals. All the cells of a given thallus are not in the same physiological and cyclogenic state, and just what controls such an evolution is not at all clear. Nor is it possible to state at what stage of development the most successful dissociative responses can be obtained. The fact that such dissociations do occur leads to the conclusion that a given spore is not a clearcut, standardized unit of known qualities, but that it is complex and is able, on germination, to change form and function and to perpetuate itself under various phases. A given organism may possess a number of phases. The eighteen strains of *F. semitectum* represent the eighteen phases in the cyclogeny of this particular organism. The writer seriously doubts if any of these and similar variants can remain immutable. Their degree of permanence should be a relative thing. Indeed, the very fact that some of them already have reverted to their original form indicates that we are dealing with the various stages of a complex organism rather than true mutations or truly new strains. On the other hand, there can be no doubt that owing to some favorable combinations of environmental factors some of these variants may perpetuate themselves indefinitely. It may be very difficult to identify life phases and true species or strains, unless each species is subjected to an intensive physiological study.

Even if one could demonstrate that food changes, bacteriophage, contamination with mites, Actinomyces, bacteria and other organisms, ultraviolet light and other waves, or any possible physical, chemical, or biological

factor or combinations of such factors might be the direct cause of mutations in fungi, this would still be of little significance taxonomically, because a given stimulus is valuable only in so far as it is able to awaken the hidden possibilities of the protoplasm. If there is nothing there to be awakened, then the stimulus itself remains as wasted energy unless it can be definitely shown that environment can put something into the protoplasm that originally was not there. The environment, therefore, can only hasten the appearance of the properties of the living protoplasm, just as X-rays probably hasten the mutation processes in the fruit fly or in the tobacco plant.

It may be said that under the "abnormal" conditions of the laboratory, where a fungus in pure culture finds no competition for food, and where the nutrient substances are in an entirely different and a more available form than are those found in nature, the fungus is more apt to find the necessary stimulus for the development of variants, and furthermore that, whereas in nature many such new forms would perish in the struggle for existence, under the tender care of the investigator they can be easily perpetuated. But, on the other hand, if an unusual stimulus is the cause of such variations (and the effect of X-rays and ultraviolet light in some animals, plants, and fungi seems to indicate that such might be the case) then where is such a stimulus more apt to be found, in the carefully controlled and simplified laboratory conditions or under the infinitely more complex natural conditions? When in a plate of agar a fungus colony develops sectors, it cannot escape the attention of the investigator. But, when such sectors are formed within the tissues of the host plant and are accidentally isolated in pure culture, they may be taken for distinct strains and their immediate relation to their parent types may not be suspected. Because the species concept in many of the lower organisms is based on too few and too indefinite characters, the investigator is very apt to go astray and take the different phases of a given species for new varieties at least. We have seen that dissociative changes affect a great many morphological and physiological characters which have been used in taxonomy as reliable criteria for differentiation. The writer regrets that he has been unable to find time for a careful study of the microscopic characters of the spores of the Fusaria, but he sees no reason why the spores should remain unaffected by dissociations when everything else seems to be more or less definitely altered. He has demonstrated that at least in case of *Phytophthora omnivora* the shape and size of sporangia are modified to such an extent in the so-called mutations as to make the new strains appear like species entirely different from their mother forms. But because the relationship of these strains to their prototype was known to the writer, he escaped the anomalous situation of having described as two species the strains of one organism. If the variants of Fusaria form an exception in which the shape and size of the spores is unaffected, they exhibit a rather singular biological behavior.

The inconsistency of physiological reactions as shown in all of the foregoing tables offers a better illustration of dissociative functions than the phenomenon of sector formation alone. When, for instance, three pieces of mycelium of equal age and size, obtained from the same colony and transferred to three different test-tubes, each tube containing the same quality and quantity of nutrient solution, yield three different kinds of reaction, it is very difficult to conceive of mere fluctuations as the causal factors. For example, what induces growth in one of the pieces of the mycelium, what is responsible for the total inhibition of the second piece, and why should the third piece be killed? And when the experiment is repeated, and the three similar pieces of mycelium are transferred to three test-tubes containing the same quality and quantity of nutrient solution, why should diametrically opposite results follow? Such conflicting and criss-crossing reactions have been listed by many dozens throughout this paper, and the experiments have been repeated time and again in duplicate and triplicate in order to eliminate all possible experimental error. Yet the same extreme fluctuation has constantly occurred, and it is not at all likely that such erratic responses could be caused by some oversight or mistake in laboratory procedure.

In addition to the experiments listed in this paper, a great many biochemical tests were conducted with these organisms, and the results obtained were so much more inconsistent that the writer was obliged to limit his work to comparatively stable tests. When variations in the vital phenomena of organisms occur under conditions as nearly controlled as possible, any explanation based on the theory of mere fluctuations is highly inadequate. If a portion of mycelium from the same colony grows readily in the presence of some inhibitive substance while another portion not only fails to grow but is killed, there is no doubt that the two pieces are not in the same physiological state but represent fairly distinct phases in the life of the colony. Dissociations of this type are readily induced by any substance which is capable of exerting an unusual or unfavorable action, as can be seen throughout the tables in this paper. We shall soon see that there is a very close parallelism in this respect between the behavior of Fusaria and that of a number of species of bacteria.

There seems to be a critical concentration of growth-inhibiting substances or factors in which the vital reactions barely balance themselves, and, dependent upon their physiological state the organisms either succeed in overcoming antagonistic action and grow, or are either inactivated or killed. That the quality and the efficiency of an organism is not uniform throughout the day is a well-known physiological fact, but as to what controls such variations, we are still in the dark. It is possible that not even the different parts of the protoplasm of a single cell are in the same state of development, because otherwise how can a single spore grow and dissociate

into different sectors and produce two, a dozen, or eighteen strains. A "pure" species, therefore, as we understand it, is a myth, because it may be composed of a great many phases which may be expressed under the action of the proper stimuli, and depending upon the environmental conditions, may either remain comparatively stable, or else may exhibit the sort of fluctuations that we have witnessed in the foregoing tables. The internal factors, which by their different combinations bring forth the specific characteristics of certain fungi, may be undergoing constant segregations and recombinations within the same protoplasm. At one time, due to one kind of combination a given cell may exhibit a characteristic which was not apparent before. The rapid proliferation of such a cell may produce sectors different from the rest of the colony, or give physiological responses unlike those of the mother type. Later, there may follow other segregations or recombinations, with the result that a reverse action follows and the old or a new type of growth may appear. This means that in reality an organism neither loses nor gains any characteristics, but because of a constant state of flux in the internal environment of the protoplasm, the different facets of the same organism are apt to appear or disappear depending upon the proper stimulus on the one hand and the evolution of the proper physiological state on the other. Perhaps many bona fide mutations, especially bud sports, result from this type of change in the internal environment of the protoplasm. Perhaps not even true mutants, if unprotected by selection, can remain immutable. Perhaps mutations are nothing more than variations or cyclogenic phases that have "jumped" a little farther.

The writer cannot believe that in all cases the different cyclogenic phases of a given species or strain lie dormant in the protoplasm until the proper stimulus comes along to activate them. It is probable that a colony which to the eye may appear purely uniform, or when subjected to laboratory tests may yield uniformly consistent reactions, may be, in reality, complex, and therefore "impure." There may be a decided masking effect operative within the colony. For instance, we know that upon opaque media such as oatmeal, sector formation is less common than upon transparent media; that thin layers of agar in the petri dish seem to induce sector formation while thicker layers fail to do so. Transparent or thin agars by themselves do not constitute stimuli, but because of their physical condition they aid the eye of the investigator to detect minute deviations from the dominant type. Sectors are not invariably clear-cut, nor can they always hold their own and grow into large sectors without being arrested or outgrown by the mother colony. Some media induce rank aerial growth, and when a sector with no aerial mycelium begins to appear, it is soon "smothered" under luxuriantly vegetating aerial hyphae. Such starchy substances as rice which induce pigment formation in the great majority of Fusaria are very poor substrata

upon which to search for pigment forming and non-pigment forming segments, whereas synthetic or semi-synthetic agars which can induce pigment formation in some varieties and hinder it in others, admirably serve for such a purpose. In reality, therefore, sectors with weak or no pigment forming tendencies may and do occur on steamed rice, on oatmeal and other agars, only they escape notice because of the masking effect of the substratum. Similarly, slow growing sectors may and do occur in the more rapidly growing colonies; irregularities in the margin of the colonies, deep clefts and heart-like effects are often caused by such slowly growing regions, and unless immediately isolated, they will soon be surrounded with and lost in the more rapidly proliferating regions of the colony. The writer has isolated a number of such slowly growing strains from a fast growing culture of *F. moniliforme*. Characteristic patterns in the margin of colonies may also be isolated and perpetuated if detected and transferred before they become again lost in the fast advancing margin of the mother colony.

When a piece of inoculum is transferred into a solution containing some more or less toxic substance, it either grows or does not grow. When growth does occur, we assume that every cell of the inoculum withstood the effect of the toxic substance. But such is not always the case, as a microscopic examination of the inoculum, shortly after a growth begins to appear, will reveal the interesting fact that many of the hyphae are killed. A similar phenomenon will be manifested if a vigorously growing mycelium is transferred to such a solution, kept there for two or three days, and examined again. When the inoculum fails to make any growth in the presence of a toxic substance, we assume that it is either inactivated or killed. But a microscopic examination may often show scattered pieces of turgid hyphae among others with advanced stages of plasmolysis. If retained in culture long enough such an inoculum may regenerate into a vigorously growing colony. It is quite apparent that not all the cells of the same mycelium are physiologically identical, and that if we could accurately separate them, new phases in the life of a given species would be revealed. Similarly, when a certain strain of bacterium brings about agglutination, we cannot say that every individual cell of the bacterial colony is endowed with such an ability, just as we cannot say that in case of a negative result every individual cell of the colony was devoid of this power; else how can a culture become spontaneously agglutinative at one time and then fail of agglutination at another? The masking effect of the sheer weight of numbers is probably a factor in such cases; perhaps very few cells in a colony may possess the ability of agglutination, but their activities remain too limited and crowded to become noticeable. The same holds true in case of pathogenic relationships whereby disease producing and harmless phases follow each other in the life of an organism.

The secret of "inducing" dissociative phases does not lie only in bringing the organisms in contact with proper stimuli, but also in being on the lookout for small and seemingly insignificant deviations in the colony habit of a given fungus. A few different agars, thin and transparent layers of petri dish cultures, and as many cultures as possible are the most important beginning points. When colonies begin to form, they should be examined daily, and any region showing the slightest variation from the mother colony should be transferred to another culture dish and watched. Once a culture begins to "break up" it may continue until many "new" strains are produced. The writer proceeded to "induce" dissociations in a strain of *F. moniliforme* which had behaved quite regularly and showed no apparent sectors; two months later 50 distinct strains were isolated from this one monosporic culture; some of these are so obviously different in appearance and behavior as to fool the most conservative taxonomist concerning their origin. Even a "pure" colony, therefore, is often an aggregate of different phases and potentialities which can be separated from one another and kept for varying lengths of time.

It may be said that if the newer phases cannot be perpetuated without the tender care of the investigator, and the "normal" type will usually assert itself, there is no necessity of separating them and thus complicating the work of the taxonomist. Unfortunately we have numerous instances where the newer phases outgrow the old. Confusion can be averted only when all the possible dissociative phases of a given strain are brought to light and studied.

The work of Löhnis and Smith (6) on the life cycle of bacteria shows many points in common with the dissociation phenomena in Fusaria. They found that a given Azotobacter can develop a great number of unbelievably diverse morphological and physiological characters, and that such radically different types may appear one after the other during the lifetime of the organism. Perhaps these authors were dealing with the effect of bacteriophage, but that does not change the fact that even a bacterium is very complex in nature, possessing many phases and cycles in its life. Smirnow (9), Kan-Ishiro (5) and a great many other bacteriologists have conducted careful experiments with variations in bacteria, and have demonstrated the existence of a great complexity in the life cycles of pathogenic microbes. Rosen (7) has reviewed much of the literature concerning variation in bacteria, and, in addition, has studied this phenomenon himself. He calls attention to the fact that the conditions provided for the so-called normal growth of bacteria often preclude the possibility of obtaining other structures which might develop in the same species under more natural conditions. Nevertheless, he points out that variations may occur not only because of the appearance of different phases in a given life cycle, each with

its own set of forms and functions, but also because of racial or clonal differences within a species.

The most thorough review of the work on bacterial "mutations" and dissociations is that of Hadley (3) who cites 518 references bearing directly or indirectly on this subject. In addition, he has done a considerable amount of work himself, and therefore more than a passing reference should be made here to his publication, especially in view of the fact that there is a remarkably close parallelism between the behavior of a great many species of bacteria and that of the Fusarium species described in this paper. Extensive and intricate cases of bacterial instability have been accumulating for the past three decades, and it seems that pure-line colonies of many bacterial species are not composed of identical cells, as the orthodox school of taxonomy would lead us to believe, but that they contain certain new strains, bearing no resemblance to one another or to the parent type. Undoubtedly a biological mechanism backed by microbial heredity lies at the bottom of such dissociations. The new strains which make an appearance may persist for a time in an apparently stable state, or may become transformed into still another type, or may revert to the original form. Thus, it has been observed that cocci may become rods and rods may become cocci or spirals, that forms of colony growth change overnight, that motility is lost or regained, that fermentation reactions are modified by time and opportunity, that spore formers lose this ability, that hemolytic activity comes and goes, that capsulated bacteria lose their capsules, and capsules are gained by noncapsulated forms. Again it has been observed that antigenic power vanishes and reappears, that cultures spontaneously become agglutinative or fail of agglutination, that virulent cultures become harmless and harmless cultures virulent, that microscopic forms become ultramicroscopic and presumably change into a virus type, and that any of these new types of behavior may become more or less permanently fixed. These dissociations or "mutations" may be enhanced by the employment of such factors as aging, changes in acidity of the medium, temperature changes, physical and chemical variations in food substances, presence or absence of oxygen, antiseptic compounds or dyes, microbial associations, the influence of specific blood or serum, body secretions and excretions, metabolic products of other organisms, and finally the bacteriophage. Hadley reasons that since such a multitude of diverse factors is capable of bringing about dissociative phenomena among bacteria, it is probable that there is a common factor in bacteria directly influenced by such environmental changes, and he indicates arrested growth as such a condition. Consequently, any environment or substance antagonistic to the continued growth of the so-called "normal" type of an organism may induce dissociations. For instance, the rod phase of the Shiga bacillus is susceptible to bacteriophage whereas the coccus phase is

not, and when attacked by the ultraparasite, the bacillus phase disappears and the coccus phase emerges to carry on the life activities of the organism. Similarly old age, high and low temperature, toxic substances, etc., merely serve to suppress one phase in the cyclogeny of the organism and release the other. A given quality, be it morphological or physiological, may lie dormant or only slightly active in the protoplasm of the microbe for lack of the proper stimulus, but as soon as opportunity arises, this hidden and hitherto unsuspected quality expresses itself. Such changes may last for only one, two, or a few generations and go back to their dormancy again, or else they may be more or less permanent as has been demonstrated by many bacteriologists and as seen in Fusaria.

Knowing the possibility for dissociations of far-reaching significance, it is the duty of the investigator not to avoid the employment of the necessary stimuli, as some suggest, but rather to make full use of such stimuli. We may hand-pick our laboratory conditions, but we cannot exercise an effective supervision upon the behavior of fungi in nature where all sorts of physical and chemical conditions are in a perpetual state of turmoil. We may never be able thoroughly to know any one species, but the only way to gain a working knowledge of the "private life" of an organism is to stimulate the emergence of all of its phases and hidden facets.

Some of the conclusions drawn by Hadley are very pertinent and applicable to mycology. The writer is so heartily in accord with them that he cannot refrain from quoting a few. Speaking of the "normal" bacterial type Hadley states that "through long years of laboratory studies we have come to regard as the normal type that form of the organism which grows best for us on artificial culture media . . . 'normal culture' or 'normal type,' in the absolute meaning of these terms and as commonly employed, is something of a myth. The stock typhoid organisms which we maintain in the laboratory are not the same in all respects as those found in the blood or intestines, nor the same as those isolated from urine or gall bladder of typhoid convalescents. . . . Shall we regard as normal the disease form, the convalescent form or the old laboratory form? When we speak of normality or abnormality, we must make our comparison not between different stages in the cyclogeny of the strains, but between corresponding stages. In other words, we must compare S type culture with S type, R type culture with R type, and intermediate with intermediate. . . . Some of the greatest sources of confusion and error, not only in systematic but also in applied bacteriology are to be found in our well entrenched habit of attempting to make comparison between fundamentally different things, that is to say, between cultures in different cyclogenic phases of development. I believe, moreover, that until this viewpoint is changed no successful advance can be made through the commonly used methods of classification of bacterial species or

races or strains by seeking to establish 'serologic types' as an end in themselves . . . it seems to me that we have arrived at a point in the development of the science of bacteriology where much work on elaborate superstructures must be suspended until we have made more stable the foundation."

Hadley believes that the time will come when the mere naming of an organism will not mean very much in taxonomy, "for it is becoming increasingly clear that we shall never know what a bacterial species really is until we acquaint ourselves with the outermost limits of its variability. Our exact knowledge of an organism may be slight even after we have noted its morphology, recorded its measurements, registered its reactions, ascertained its common serologic and antigenic characters and given it a name." Before there is a possibility of reorganizing the present inadequate conception of the nature of bacterial species and of the systematic relationships of bacteria, it will be necessary to abandon, for the present at least, "our vain attempts to perfect schemes of classification." Furthermore, it will be imperative to initiate "a somewhat new branch of bacteriological study in which we shall strive to recognize bacterial species relationships, not by comparison of isolated single cultures of this or that, but by a study of all the various types or stages comprising the cyclogeny of the species in question —a difficult but necessary task. . . . *Bacillus diphtheriae,* as assumedly typifying a definite bacterial species, should eventually come to mean to us not merely a rod of fixed size and shape (possessing certain constant biochemical, serologic, immunologic, and staining reactions, by means of which it can conveniently be recognized and cataloged), but in reality a host of things, which we must be able to recognize individually and collectively."

Because Fusaria behave very much like the bacteria possessing cyclogenic and dissociative properties, and since the taxonomy of this genus is far from being perfect or even comprehensive, solutions offered by Hadley for the taxonomic puzzle of bacteria should be applicable to this and similar groups of fungi.

The fluctuations in vital responses which a given strain of fungus can manifest under even the most uniform environmental conditions seem to bear a pertinent relation to the occurrence of plant diseases. Why, for instance, are many diseases sporadic in nature notwithstanding the constant presence of host and parasite on the one hand and favorable environmental conditions on the other? Why may artificial inoculations fail even though the best of conditions for successful infection have been provided? Why is it that some plants are easily attacked and others show an apparent immunity? Is it not possible that the biological conditions of the parasite as well as those of the host plant may be responsible for this? Environmental factors have been constantly brought forward to answer these questions;

and, while no one can deny the great potency and the influential rôle of the environment, the biological mechanism of the parasite should not be relegated to comparative obscurity. Since dissociations enable the fungus at one time to withstand toxic substances and unfavorable factors and thus grow unhindered, and at other times to be totally inactivated or even killed, it is only logical to assume that similar conditions may, at one time, enable the parasite to overcome the resistance of its host, and hinder it from doing so at other times. Since most of the resistance offered by the host seems to be of chemical origin, it is clear that factors which control the ascendency of host and parasite in their struggle are to be found not wholly in the environment, or in the chemical and physical conditions of the host plant alone but also in the ever-shifting qualities of the parasite itself. A proper understanding of dissociative phenomena and an adequate control of the internal mechanism of fungus protoplasm seem to offer the most vital and the least explored field in plant pathology.

CONCLUSIONS

It is concluded that, notwithstanding the great variability, the inconsistency of certain morphological and physiological characters, and the appearance of a number of new characteristics in many Fusaria, a certain specific line of behavior persists throughout all of the strains and variants of a given fungus species. The path of the species may run in a zigzag line, but so far as the present experiments are concerned, it does not exceed the specific orbit.

None of the tests, when used either alone or in combination, should be deemed sufficiently dependable for the identification of species or varieties. However, if added to the more stable morphological and pathological characters, such tests may solve some of the perplexing questions in the taxonomy of fungi. The presence of distinct strains and variants within the same species, and their decidedly different reactions seem to indicate that the concept of the species must not be that of a single organism but that of a group of many organisms having in general the same principal characters.

Just as many of the physiological reactions, if used alone should not be considered specifically conclusive, similarly, because of the erratic occurrence of the types of sporulation, of different macroscopic mycelial characters, of color production, and of nonnegligible fluctuations in the size and the shape of spores, such characters alone cannot be considered dependable or of very great taxonomic value. Students of fungi should always be on guard against the pitfalls of chance variations, and should depend only upon thorough, well controlled, standardized, and repeatedly checked studies of morphology, physiology, and pathological affinities of the organism.

SUMMARY

1. It has been the purpose of these studies to trace the variability of Fusarium species and to demonstrate the continuity of the specific path which is believed to persist in all the strains of a given species regardless of numerous sharp deviations from the known specific behavior.

2. Cultures of Fusaria, obtained from various sources under 96 different specific and varietal names, were used in these studies. Some of these were originally received from two to seven different sources and for the sake of convenience have been called strains.

3. During the study many of the cultures, all of which were supposed to be pure, showed segmentations in the colonies. These were separated in pure cultures and termed variants. In some cases these variants continued to remain different from their parent types. In others there was a reversion to the original type, or a constant fluctuation back and forth.

4. About 220 cultures were studied. All were tested from the standpoint of rate of growth of colonies, production of color and spore masses on five different agars, sporulation on malt extract agar, growth at 5°, 25°, 34°, and 37.5° C., growth and reproduction in presence of nine different concentrations of tartaric acid, growth in presence of two concentrations of tannic acid, two concentrations of mercurochrome, five concentrations of malachite green, two concentrations of iodic acid, one concentration of acid phenyl acetic, two concentrations of sodium dinitrophenoxide, and three concentrations of sodium tribromphenate.

5. The foregoing tests showed that not only different species, but even some of the strains and variants of the same species manifested decidedly different reactions in the foregoing tests. However, many strains and some closely related species often showed strikingly similar responses.

6. Even under controlled conditions the reaction and behavior of many organisms are extremely erratic.

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EXPANATION OF PLATES

PLATE XVI.—*Fusarium coeruleum* dissociating (upper colony); the two strains separated (lower colonies).

PLATE XVII.—*Fusarium tirchothecioides* 2 dissociating (upper colony); the two strains separated (lower colonies).

PLATE XVIII.—*Fusarium trichothecioides* 3–I and 3–II (upper colonies). *Fusarium solani minus* I and II (lower colonies).

PLATE XIX.—*Fusarium martii* 3 dissociating (upper colony); the two strains separated (lower colonies). Very rarely was a pure colony of the blue form, as shown in fig. C obtained in petri dish cultures. For nearly two years whenever a transfer was made from this strain it gave rise to a complex colony composed of white and blue sectors. While the white form was relatively more stable, it did, occasionally, give rise to blue sectors. A number of single spore cultures gave the same kind of results. Then suddenly all blue color disappeared and has not been observed since. Transfers made from stock cultures with deep blue color yield nothing but pure white colonies.

PLATE XX.—Two colonies of *Fusarium semitectum* 1 dissociating.

PLATE XXI.—Some of the strains of *Fusarium semitectum* 1. The variant I is extremely unstable as can be seen from the first two colonies. A great number of attempts to fix the type met with failure.

PLATE XXII.—Three types of dissocations in *F. tricinctum* 1.

PLATE XXIII.—The variants of *F. tricinctum* 1. Note that variants I and VII show a complex type of colony. At present time of writing this dissociating habit has disappeared.

PLATE XXIV.—*Fusarium martii phaseoli* 2 dissociating (upper two colonies); the two strains separated (lower two colonies).

PLATE XXV.—The three strains of *Fusarium martii phaseoli* 1.

PLATE XXVI.—*Fusarium eumartii* 1 dissociating (upper colony); the two strains separated (lower colonies).

PLATE XXVII.—*Fusarium eumartii* 2 dissociating (upper colony); the two strains separated (lower colonies).

PLATE XXVIII.—*Fusarium striatum* 1 dissociating (upper colony); the two strains separated (lower colonies). The petri dish, from which the upper picture was taken, was inverted in order to show the nature of segments more clearly, hence the apparent difference in the growth habit of the colonies.

PLATE XXIX.—*Fusarium fructigenum maius* 2 dissociating (upper colony); the two strains separated (lower colonies).

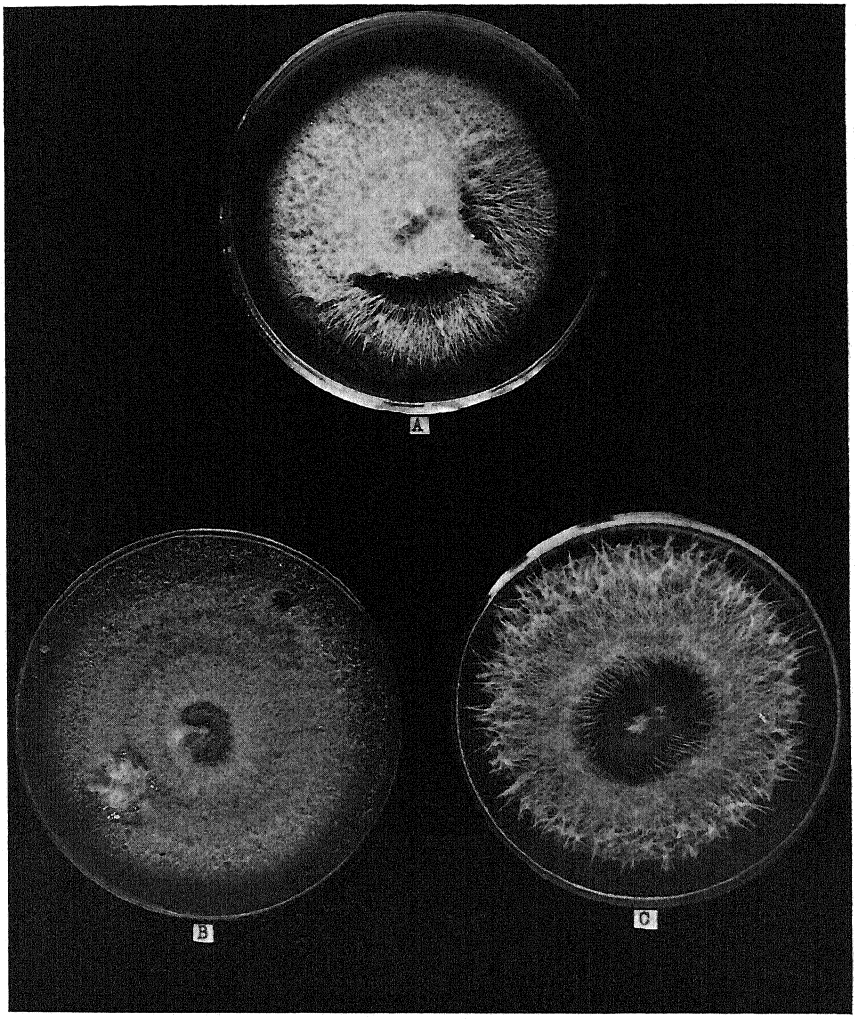
PLATE XXX.—*Fusarium culmorum* 1 showing reversible dissociations. The colo-

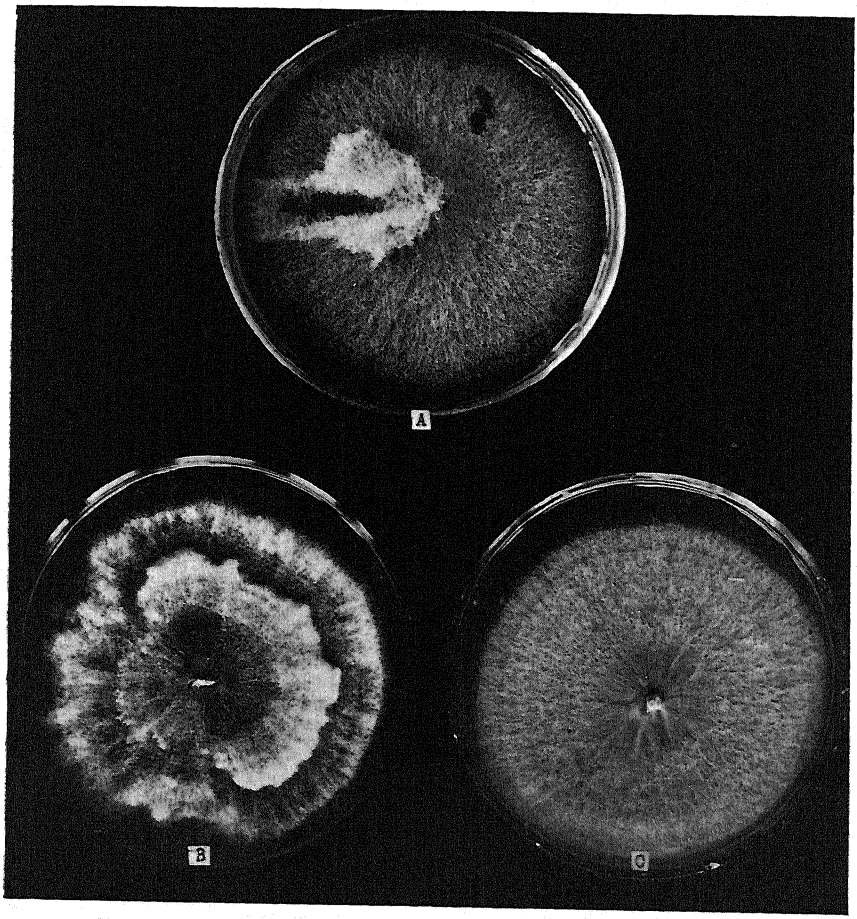
nies, shown at A and C have reverted to one another quite often, while the type illustrated by B has appeared only sporadically. All efforts to fix the types have been futile.

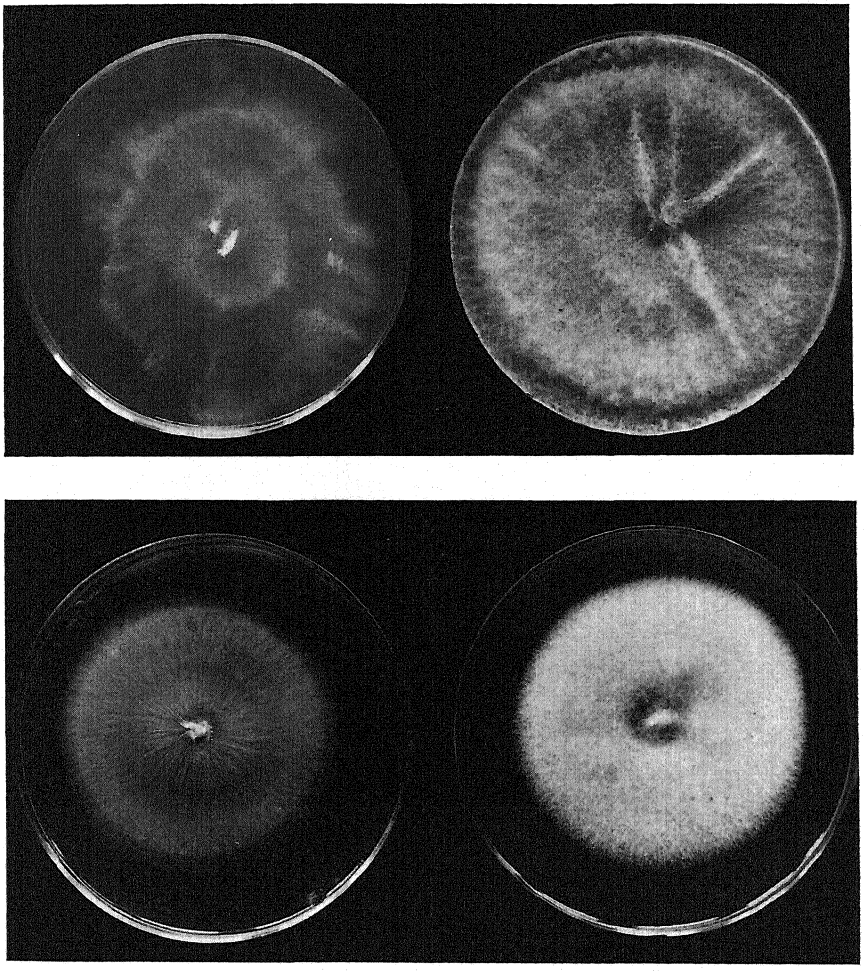
PLATE XXXI.—*Fusarium herbarum gibberalloides* showing the two strains.

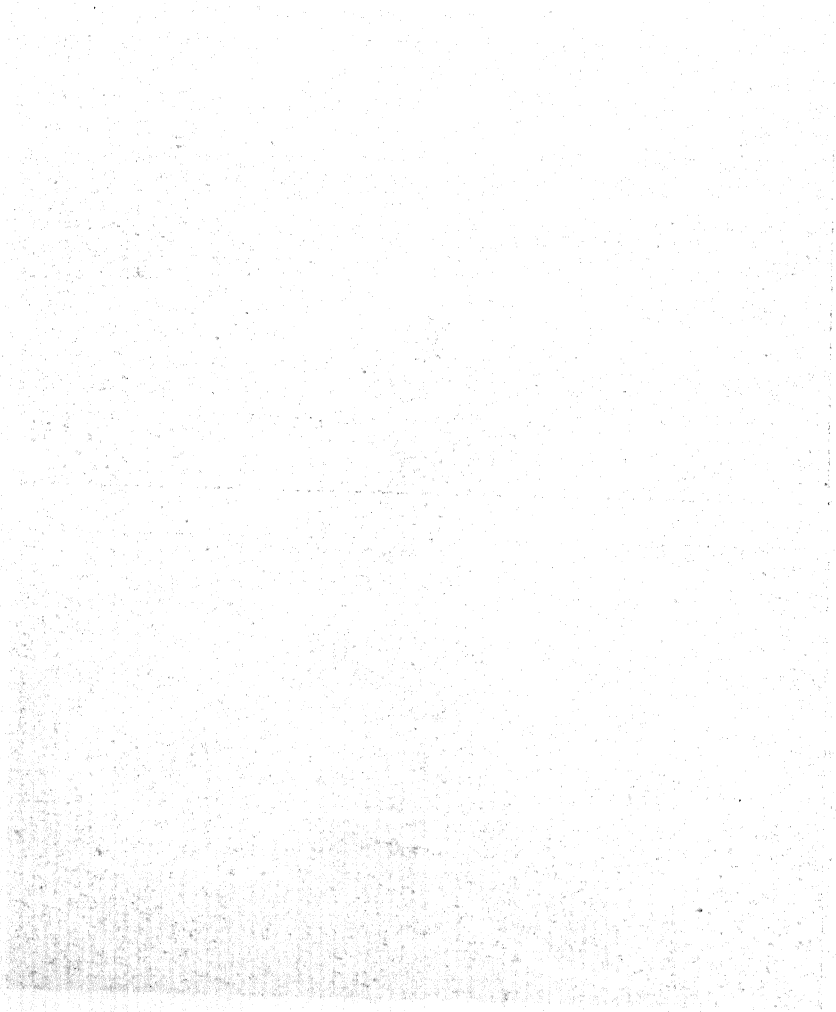
PLATE XXXII.—*Fusarium culmorum* 3 dissociating (upper colony); the two strains separated (lower colonies).

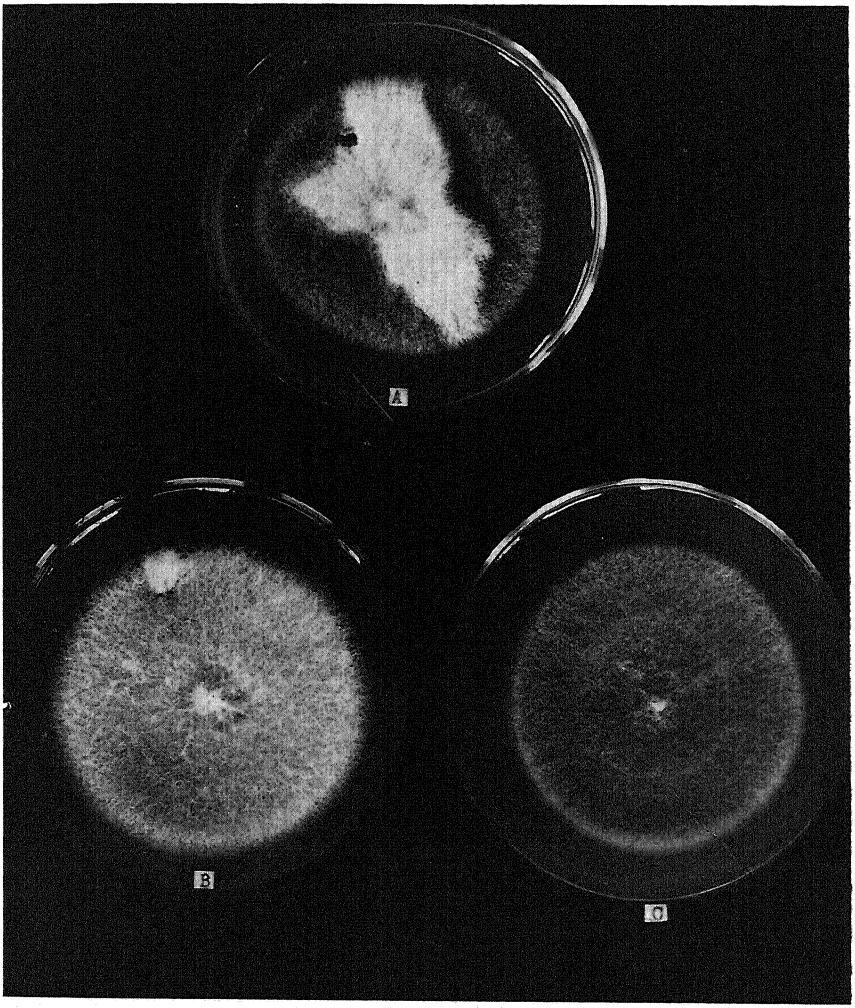
PLATE XXXIII.—*Fusarium acuminatum* 2. At the time when this picture was taken all four colonies were of the same age and had been growing under identical conditions. Note the "regeneration" of the colony at C where the white, compact growth has formed a loose and rapidly growing mycelium. Transfers made from such fast growing regions to plates of nutrient agar yielded very slow-growing colonies. These erratic growth habits conitnued for six months or so and then suddenly disappeared. Now all of the eight strains which were thrown off by the original culture are rather rapid-growing organisms.


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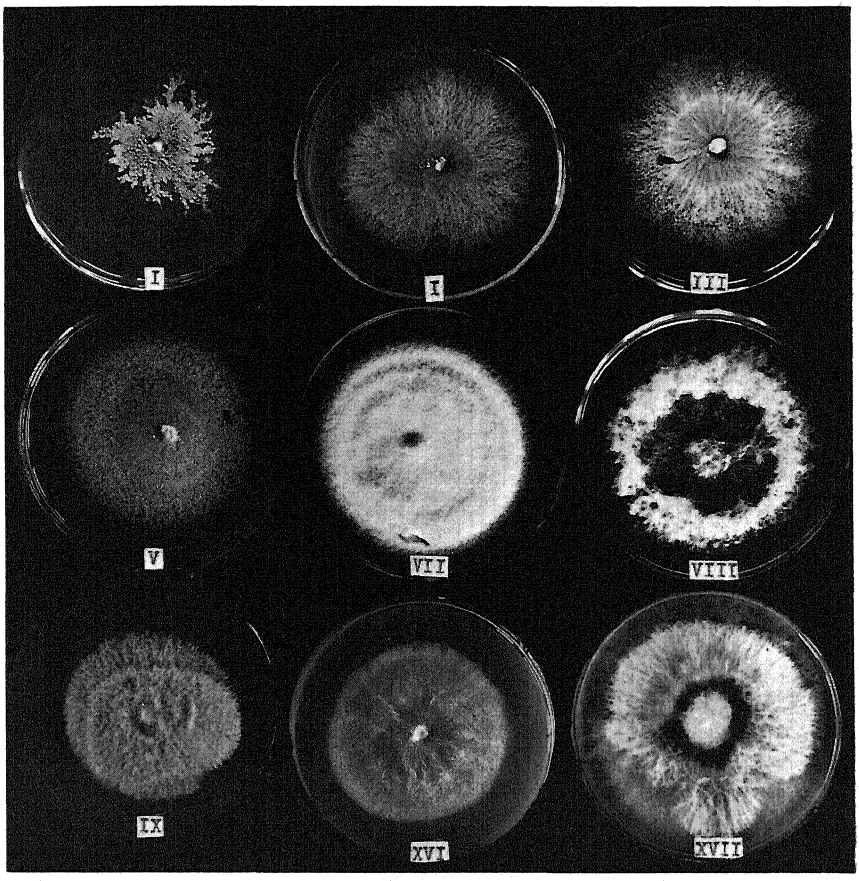

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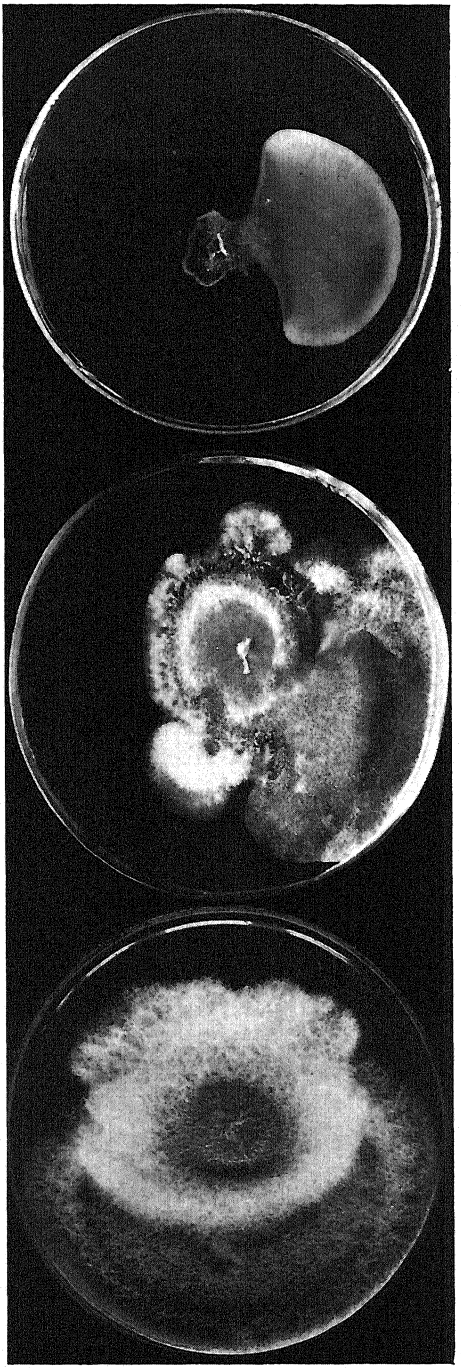


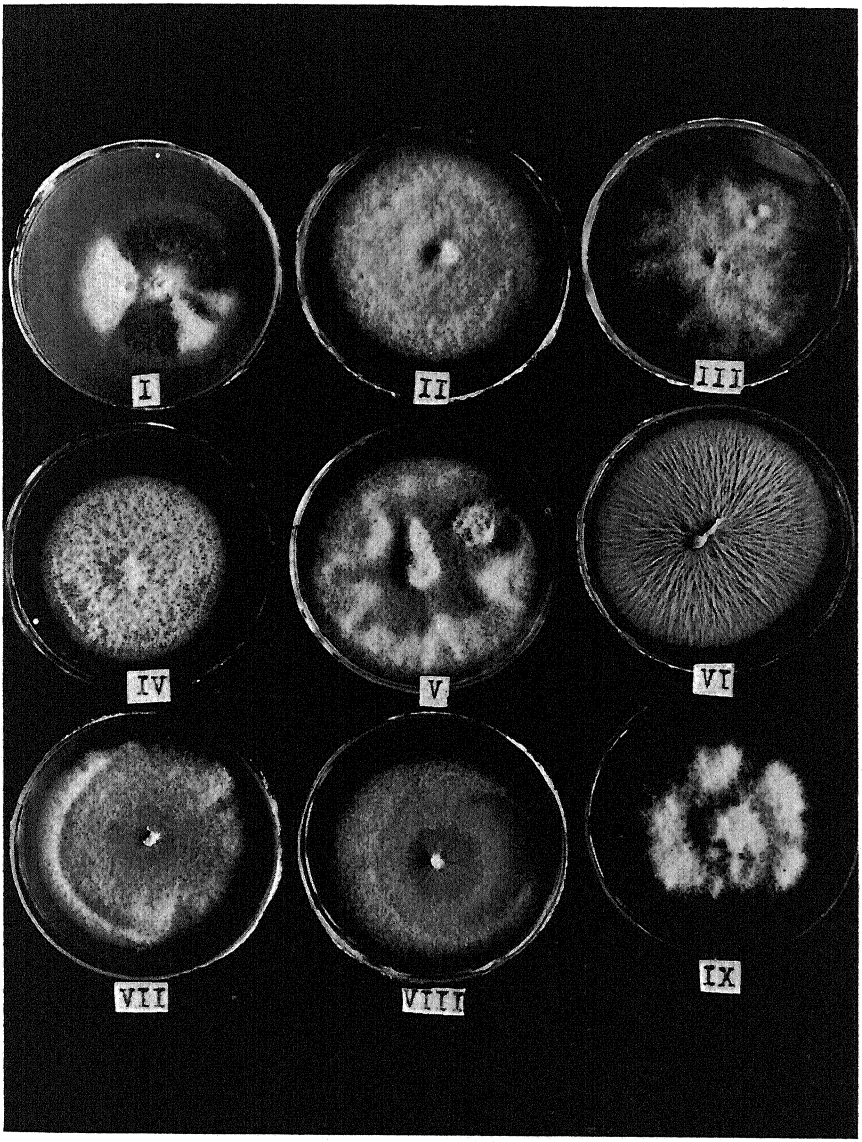


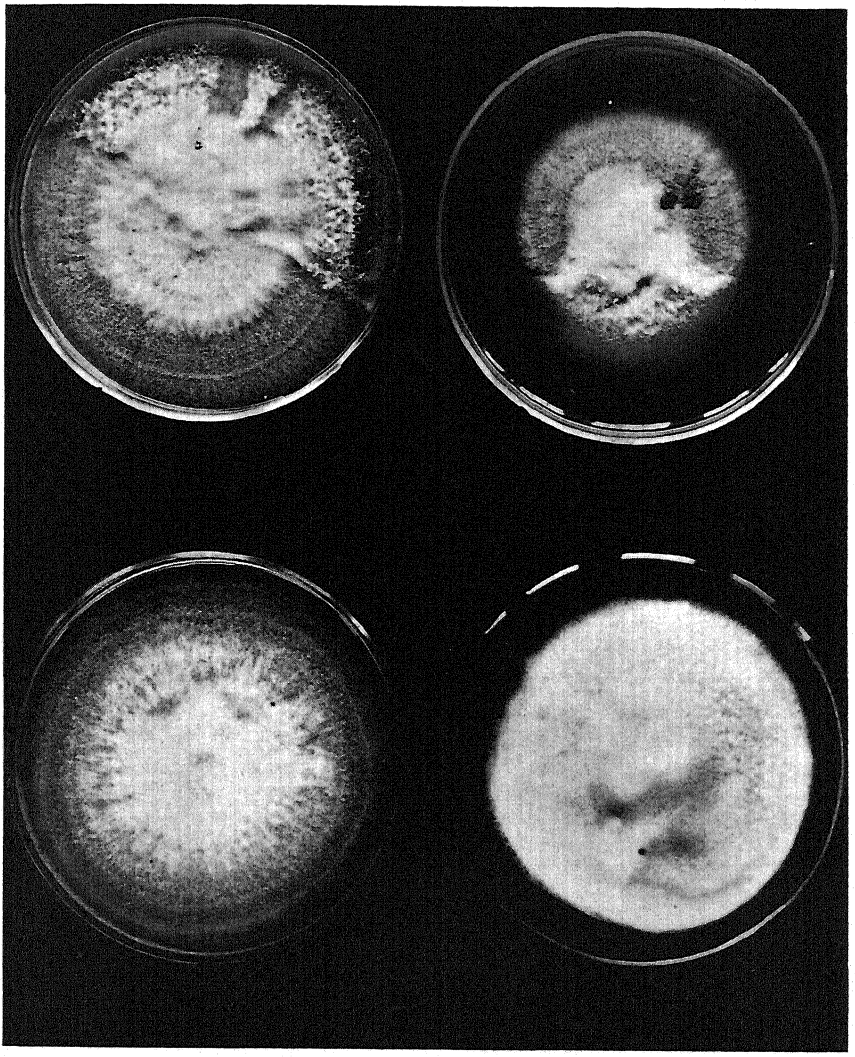

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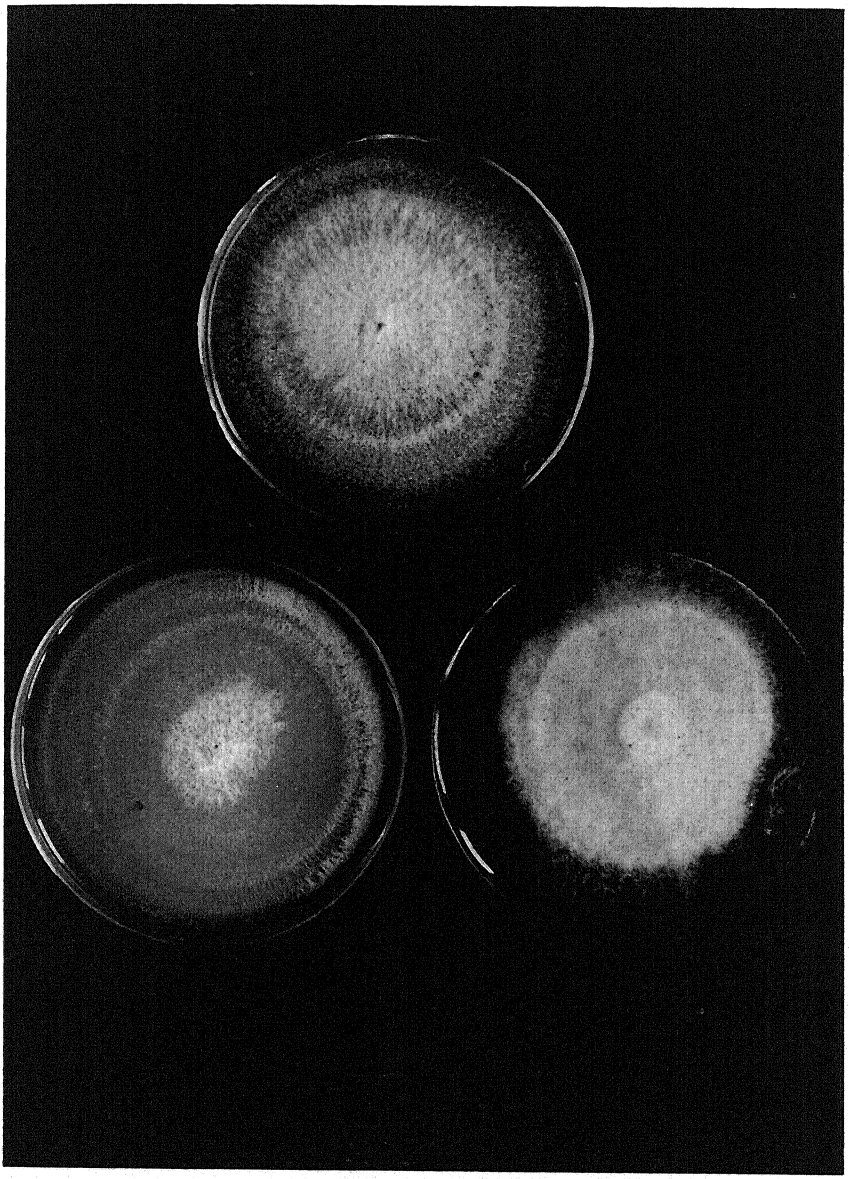


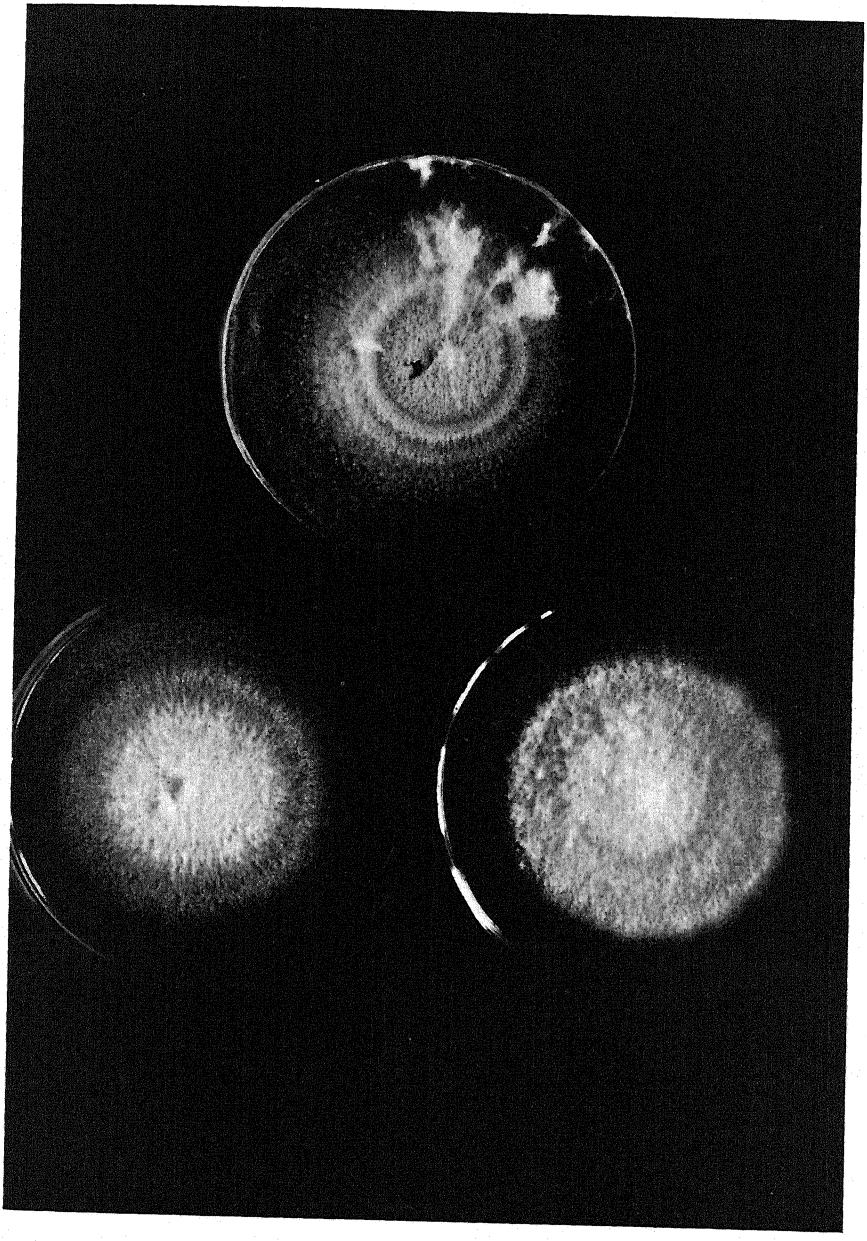

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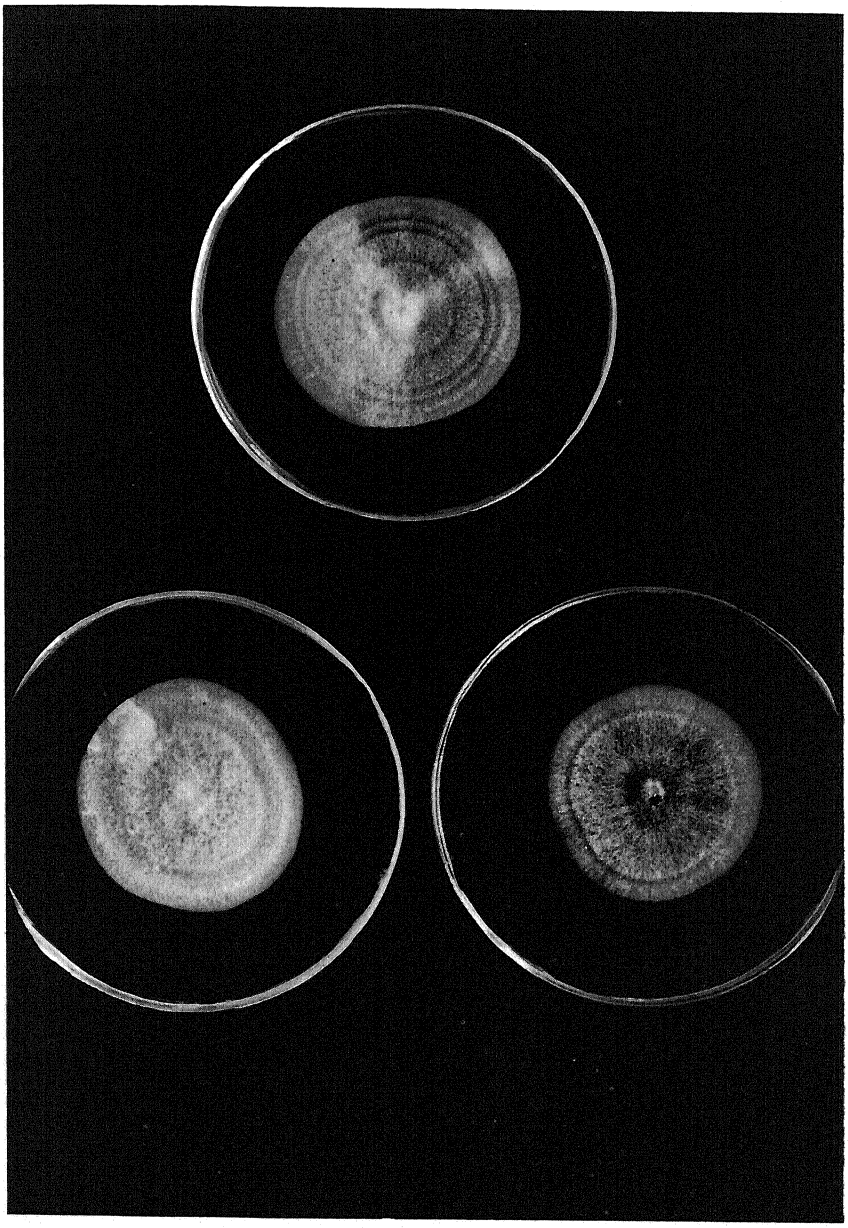

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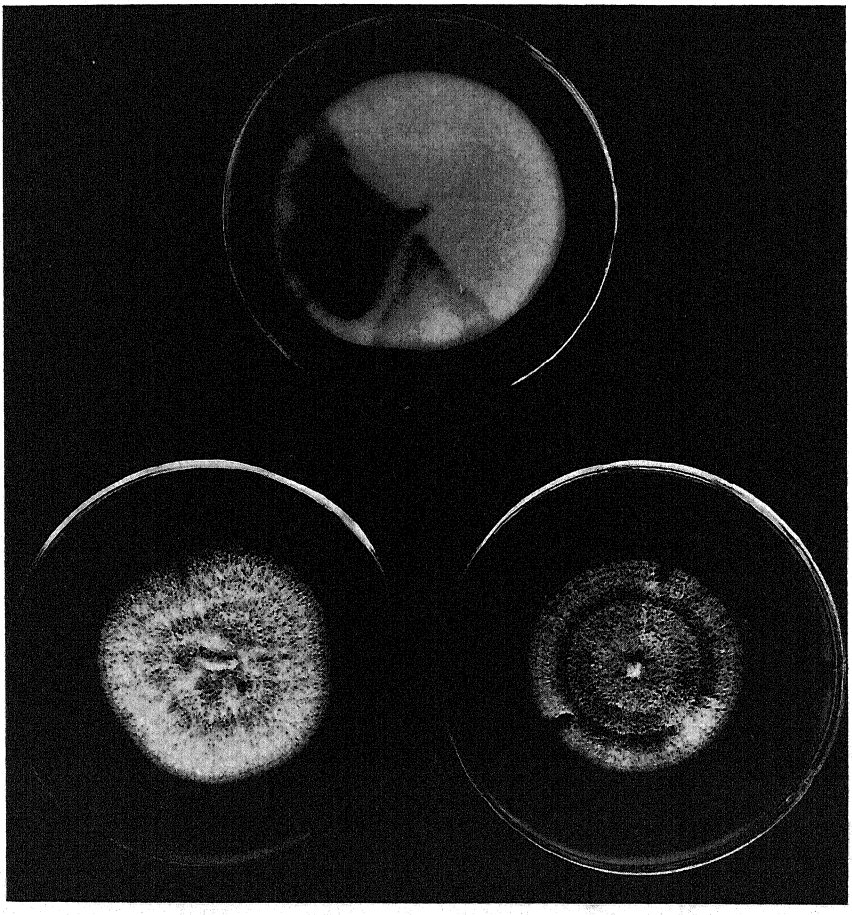




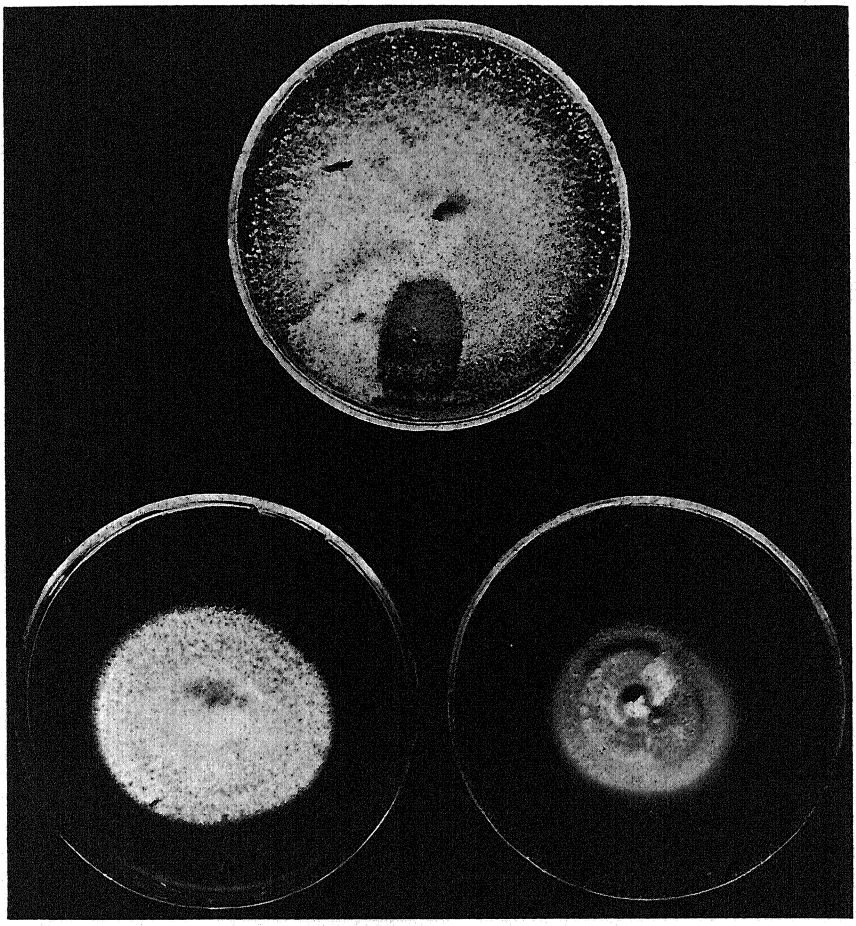




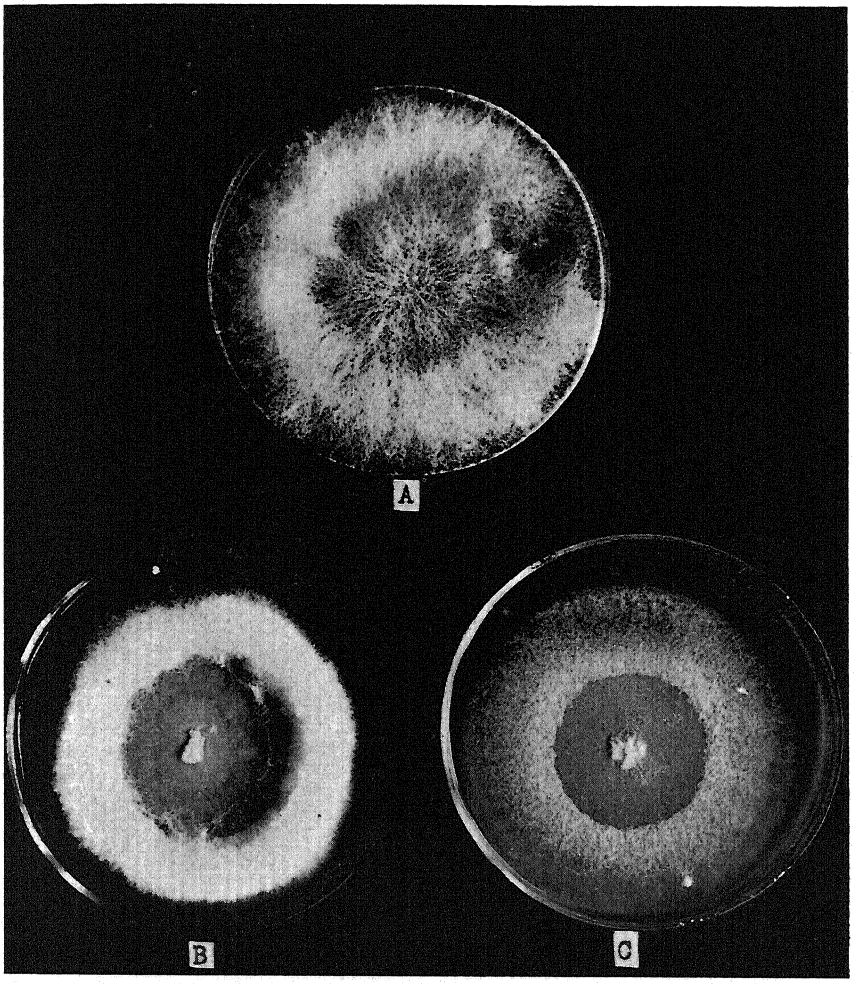


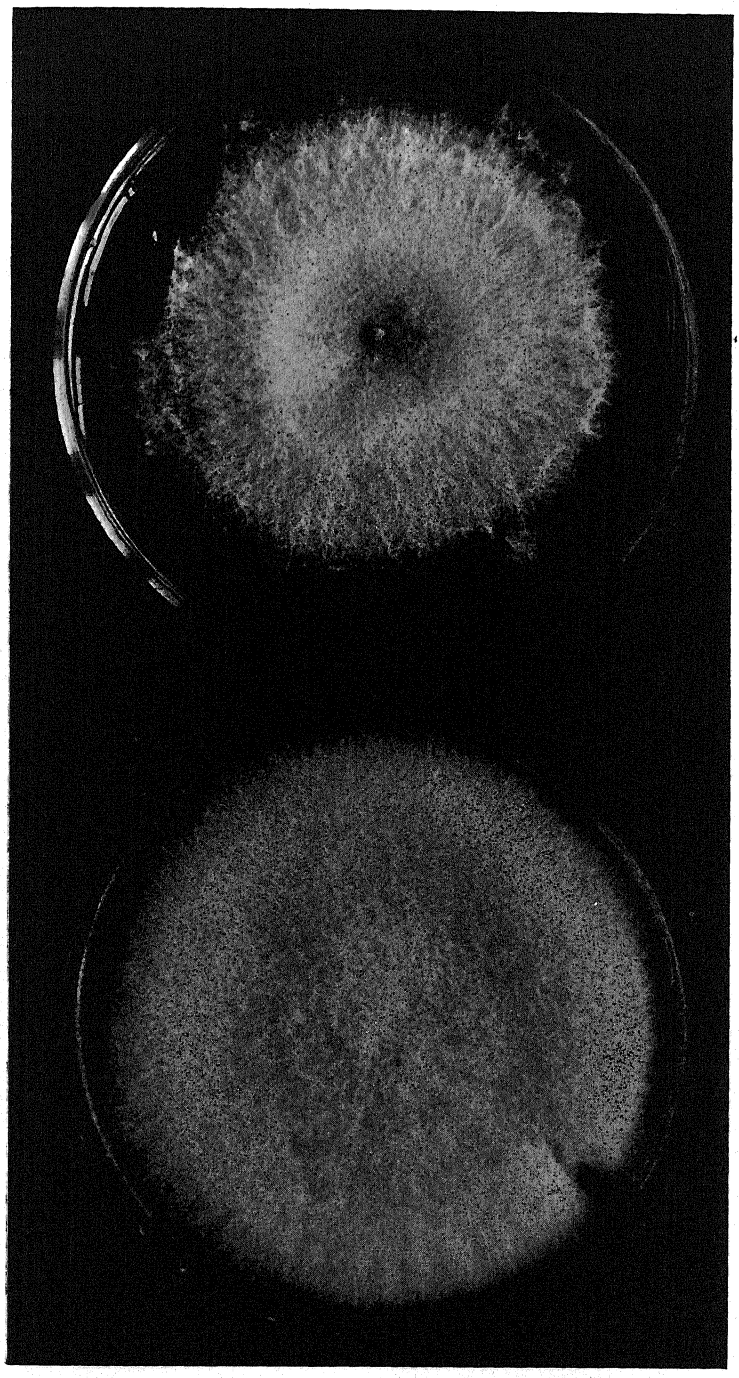


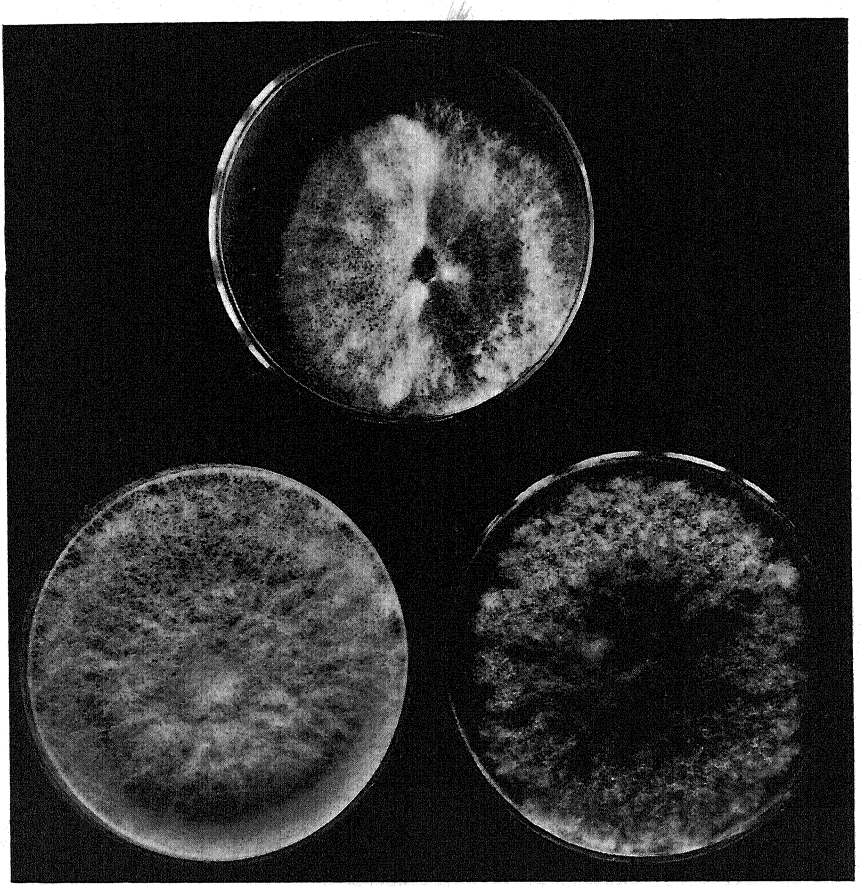


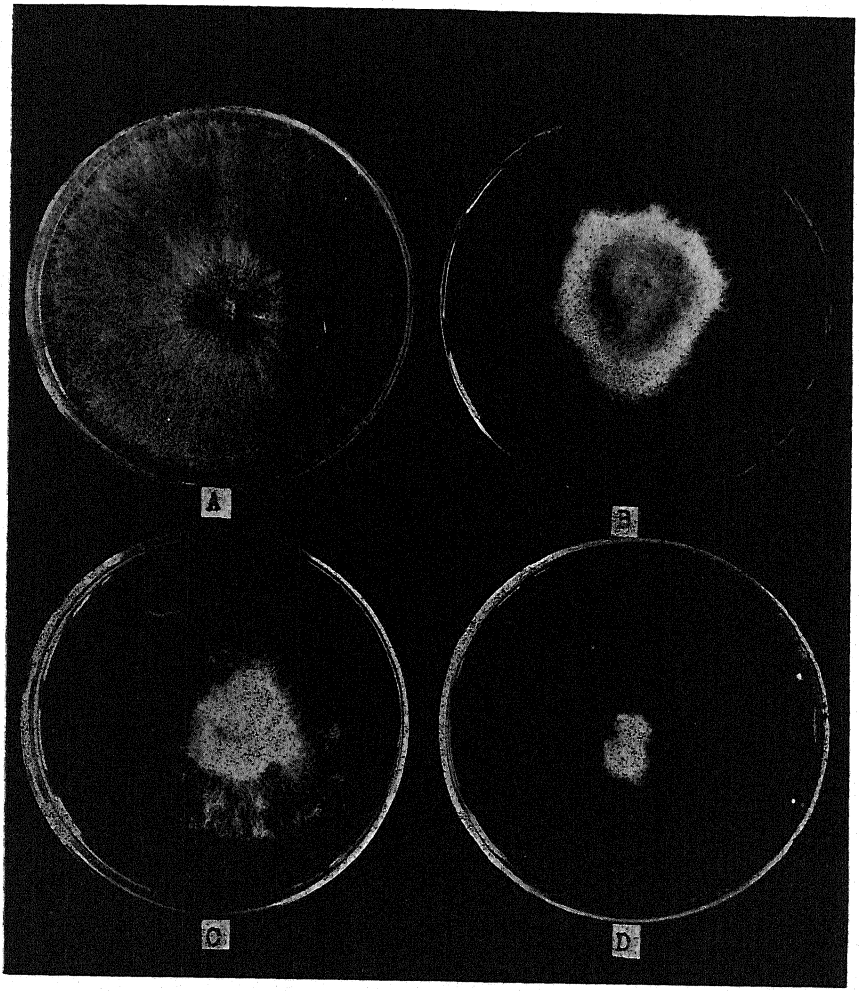





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OBSERVATIONS ON ARMILLARIA ROOT ROT OF ORCHARD TREES

LEROY CHILDS AND S. M. ZELLER

INTRODUCTION

The root-rot disease of fruit and nut trees caused by *Armillaria mellea* Vahl is very prevalent in certain sections of the Northwest; in fact, in western Oregon the fungus is much more prevalent than is indicated by the death of orchard trees. This would seem to be due to the fact that the most prevalent strain of this species apparently does not attack roots of deciduous trees. The purpose of this paper is to report observations on two apparent physiologic strains of *Armillaria mellea*. One of these, it would seem, inhabits the roots of conifers, especially Douglas fir (*Pseudotsuga taxifolia* (Poir) Britt.), in the forests, while the other strain is found conspicuously in oak-cleared land. The latter is so consistently associated with the oak in the minds of orchardists and plant pathologists of the Pacific-Coast States that this strain of *Armillaria mellea* is now commonly known as the "oak fungus." For immediate convenience in this paper, these two strains will be designated the "fir and oak strains," respectively.

THE FIR STRAIN

For several years previous to 1920 the senior author had noticed a great many orchard trees possessing practically a complete network of rhizomorphs about the larger roots and crowns. These were in every observable respect similar to those associated with trees infected with Armillaria root rot. Some of these trees were observed for a number of years. Apparently there were no infections and the trees in all such cases remained sound and maintained normal vigorous growth, giving no indication of inconvenience due to the presence of the fungus.

In the fall of 1920, some interesting observations were made in a block of the apple orchard belonging to Childs and Graff at Dee in the upper Hood River Valley. The trees at this time were 8 or 9 years old. Of the 288 trees in the block, 164, or 57 per cent, were encircled at the crown by a dense collar of sporophores of *Armillaria mellea*. Careful examination revealed that the stems of the mushrooms were solidly attached to the bark of the trees at the ground line. A network of rhizomorphs were found, not only on the crown of the trees supporting mushrooms, but on all trees examined on which there were none. Figure 1 illustrates the relation of the mushrooms to the crown of the tree and shows the thick weft of rhizomorphs on the large roots.

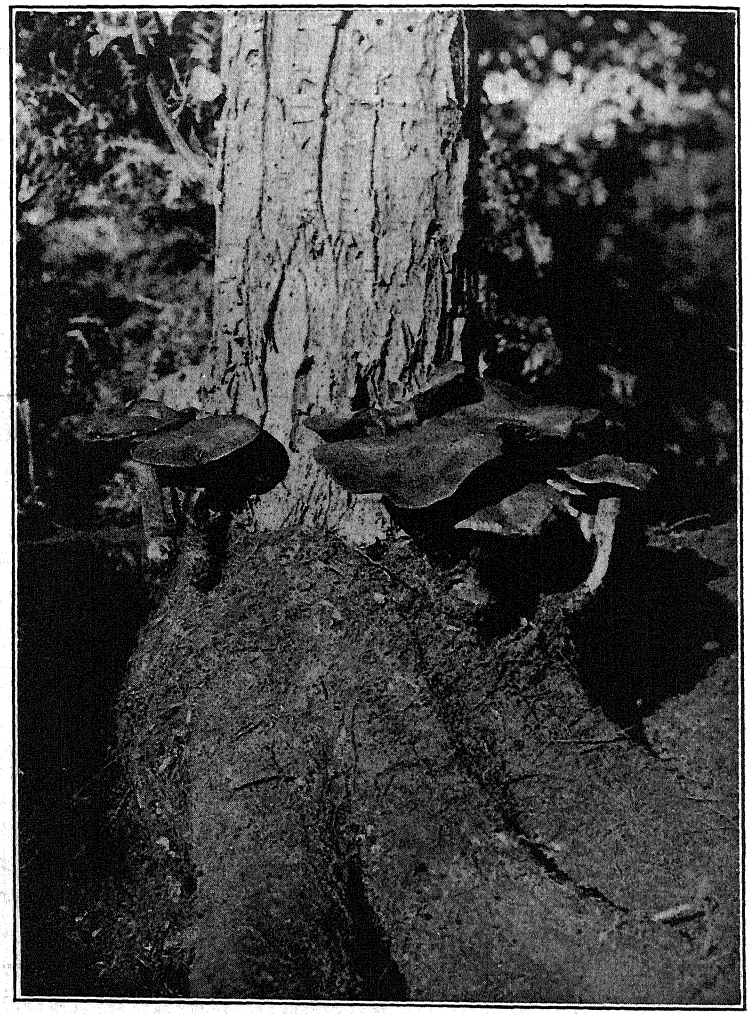


FIG. 1. Crown of an apple tree showing fruiting bodies of *Armillaria mellea* and thick weft of rhizomorphs on the large roots.

The land where this orchard was planted was virgin, fir-cleared soil. Much débris of fir wood remained in the soil. We were able to trace rhizomorphs from the roots of apple trees to fir snags buried in the soil at depths of five feet.

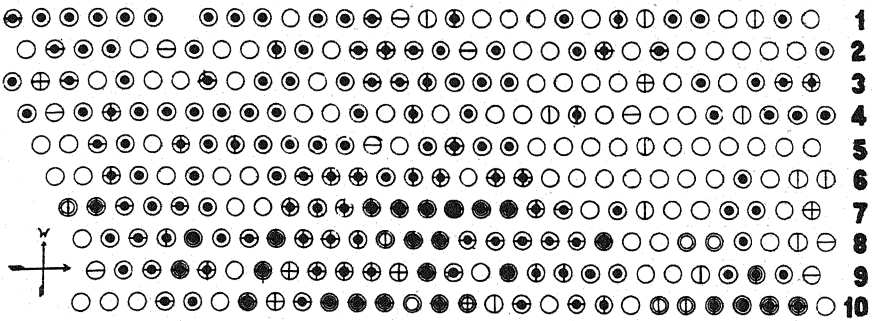
Several types of experiments were devised in the fall of 1920 to learn if wounds were necessary to permit infection by this fir strain of *Armillaria mellea*. In some instances apple roots were pruned and removed, leaving only the cut stub, while others were merely sawed off and left to decay in

the soil as an inoculum for the pruned stub. In other cases, roots were girdled by removing about two inches of bark; others were sawed to the pith and half cylinders of wood about two inches long were removed. These were again covered and observed in the falls of 1921 and 1922. After one or two years, these wounds had been thoroughly grown over by rhizomorphs but close examination revealed no infection whatever.

The occurrence of fruiting bodies of the fungus was checked on these trees from 1920 to 1924. The accompanying plot of the orchard (fig. 2)

PLOT OF APPLE ORCHARD ON FIR-GRUBBED LAND ILLUSTRATING THE PRESENCE OF ARMILLARIA MELLEA AS INDICATED BY THE OCCURRENCE OF ITS FRUITING BODIES AROUND TREES DURING THE YEARS 1920-1924.

FRUITING BODIES IN 1920=◉, 1922=⊖, 1923=◐, 1924=○



gives the number of trees bearing fruiting bodies each year. There were no mushrooms around the trees in the fall of 1921, due perhaps to the fact that the very light precipitation came late, and the first period of moisture sufficient to induce fruiting of the fungus came simultaneously with freezing night temperatures. In 1922, 1923, and 1924, there were 30, 28, and 30 per cent, respectively, of the trees surrounded by mushrooms. Unfortunately, in 1924, records were obtained on 4 rows of trees only. During the five years, 69 per cent of the trees had mushrooms of *Armillaria mellea* growing around them one or more seasons.

In the fall of 1925, when these apple trees were 13 or 14 years old, they were pulled out. The roots of all the trees were covered with a mesh of rhizomorphs but with the exception of one lone root no infections were found. In the neighboring fir forests there are occasional oak trees and it is possible that an oak sometime stood near the edge of the plot where this infected tree was removed. When the trees were pulled, they were in vigorous condition, having had an unusually good color during the previous summer.

Many other instances illustrating the saprophytic nature of this fir-strain of *Armillaria mellea* have been observed in the Willamette Valley, Oregon. These were mostly cases where one or more oak trees were known to have been present in an otherwise pure stand of fir on land now devoted to prune or walnut orchards. In the Skyline Orchards, near Salem, were found examples of mushroom root rot in which prune and walnut trees were dying in rather definite areas where oak trees were konwn previously to have occupied the land. The remainder of the orchard was mostly on fir-cleared land. The roots of many of the trees in these portions of the orchard were well covered with a network of Armillaria rhizomorphs but in all cases the trees were vigorous.

It may be that this fir strain of *Armillaria mellea,* as found in the Pacific Northwest of the United States, is distinct from that found on conifers in Europe. The following statement by Van der Meer[1] and similar expressions by other European workers would indicate parasitic virulence rather than a saprophytic nature of the strain from conifers in Europe. She says, "In 1922 the author observed the great damage caused by *Armillaria mellea* in a cherry orchard . . . which was planted on fir soil."

THE OAK STRAIN

On the other hand, in all instances where Armillaria has evidently had its source in the native oak (*Quercus garyana* Dougl.) virulent infection is the rule. Many cases could be cited in the Willamette and Hood River Valleys in Oregon. Hendrickson,[2] in California, says "it is not difficult to find, in orchards which have been long owned by one family, authentic cases where the disease has developed in areas from which oak trees had been removed many years before." He also gives the history of a typical orchard infected from oaks which were cut down some 30 years before. Undoubtedly other native vegetation also acts as a source of infection but circumstantial evidence at least would point to the virulent strain in the Pacific Coast States as having its origin in oak or similar wood.

One of us[3] recently described two modes of root infection by this oak strain, besides the entrance through wounds. Some have reported infection through graft unions but we have not observed such cases. Nevertheless, it would seem that crown infections are common in older trees where the graft unions are as smooth as any other portion of the crown. Since the crown

[1] Van der Meer, J. H. H. Verticillium wilt of herbaceous and woody plants. Med. Landbouwhoogesch. Wageningen 28: (2) 1–82. 1925. (See p. 62.)

[2] Hendrickson, A. H. Oak fungus in orchard trees. Calif., Agr. Exp. Sta. Circ. 289–13 p. 1925.

[3] Zeller, S. M. Observations on infections of apple and prune roots by *Armillaria mellea* Vahl. Phytopath. 16: 479–484. 1926.

gives rise to many adventitious stem and root buds, it may be that the crown infections occur through the points of their emergence, as previously described.[4]

In conclusion, it should be said that macroscopic or microscopic examinations and comparisons of the sporophores from the oak and fir strains of Armillaria reveal no appreciable differences in morphology, color, etc.

SUMMARY

This paper presents the histories of typical cases of *Armillaria mellea* on roots of orchard trees planted on (1) fir-cleared land and (2) oak-cleared land. There is evidence of two distinct physiologic strains of the fungus. The oak strain is parasitic, while the fir strain apparently is not.

OREGON AGRICULTURAL EXPERIMENT STATION,
CORVALLIS, OREGON.

[4] Zeller, S. M. *l.c.*

THE EFFECT OF GRAFTING ON RESISTANCE AND SUSCEPTIBILITY OF BEANS TO *COLLETOTRICHUM LINDEMUTHIANUM*[1]

J. G. Leach

In an earlier paper (3) on the parasitism of *Colletotrichum lindemuthianum* (Sac. and Mag.) Bri. and Cav. the author described the development of the fungus in resistant and susceptible hosts and concluded that the scant growth and subsequent death of the fungus in a resistant host were due to starvation. The observed phenomena seemed to indicate inability of the fungus to utilize the food materials elaborated by the protoplast of a resistant plant, while it could readily utilize that elaborated by a susceptible host. However, it was shown that the fungus would grow equally well on sterile, unheated, expressed sap of either resistant or susceptible hosts. This would indicate that the cell sap was essentially modified by extraction or that the inability of the fungus to utilize it before extraction was due to the manner in which it was held by the living protoplast rather than to the specific nature of the sap, itself.

Hoping to throw additional light on the problem, it was thought advisable to graft resistant and susceptible varieties and to observe the effect on resistance and susceptibility. Owing to pressure of other work, it was impossible to complete this phase of the work until quite recently. In the meantime, the effect of such grafting on susceptibility and resistance to other fungi by other hosts has been reported by several workers. Roach (5, 6) made reciprocal grafts of varieties of potatoes resistant and susceptible to *Synchytrium endobioticum* (Schilb.) Perc. He found that the nature of the scion had no effect on the resistance or susceptibility of the tubers developing on the stock. Plants grown from such tubers likewise were not altered in their reaction to the parasite. This work has been extended and verified by Köhler (2). Similar results were obtained by Salmon and Ware (7) who made reciprocal grafts with varieties of hops (*Humulus lupulus* L.) resistant and susceptible to hop mildew [*Sphaerotheca humuli* (DC.) Burr.].

On the other hand, Wormald and Grubb (8) presented evidence to show that when an apple scion, susceptible to crown gall, is grafted on a stock of a resistant variety the stock loses its resistance and becomes susceptible.

Hofmann (1) has made reciprocal grafts between several varieties of beans and between *Phaseolus vulgaris* L. and *P. lunatus* L. He found that

[1] Published with the approval of the Director as Paper No. 841 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The major portion of these experiments was carried out at the Biologische Reichsanstalt, Berlin-Dahlem, Germany, while the writer was a fellow of the International Education Board. The writer expresses his appreciation to the International Education

the longevity of the rootstock of *P. vulgaris* was increased by top grafting with *P. lunatus*. There was no evidence of the translocation of anthocyanin pigments from one graft symbiont to the other. Richmond (4) presented evidence to show that when a lima-bean top is growing on a navy-bean root a pure culture of the lima-bean strain of nodule bacteria will not form nodules on the roots of the grafted plants. On the other hand, when seeds are produced on such a plant or the reciprocal graft, they are so modified that plants grown from them develop nodules when inoculated with pure cultures of either strain of the nodule bacteria.

In the experiments reported here, two varieties of beans (*P. vulgaris*), one susceptible (Ruby Horticultural Bush, No. 21), and one resistant (Large Garapata, No. 20), to a physiologic form of *Colletotrichum lindemuthianum* were used. Reciprocal grafts were made soon after the first pair of true leaves were fully expanded. Two plants, one of each variety, were selected with epicotyls having approximately the same diameter. The epicotyls were severed with an improvised knife made from a Gillette safety-razor blade. A long, downward-sloping cut was made from each side, about half way between the top of the hypocotyl and the first pair of leaves, thus giving the scion a wedge-shape end which engaged a corresponding cleft in the stock. The scions were interchanged and loosely held in place with a small cotton thread. The entire point of contact was then painted over with melted paraffin applied with a camel's-hair brush. The plants were finally placed in a moist place for further growth. With care and a little practice a fairly high percentage of successful grafts were obtained.

Owing to the somewhat retarded growth of the scion, the latent buds just above the cotyledons usually began growth so that after about two to three weeks there was a set of developing shoots both on the stock and on the scion. These were then inoculated with the spores of *C. lindemuthianum* and incubated in the usual manner. Normal plants of each variety were always inoculated at the same time. In all, about 15 plants of each type of graft were inoculated. In no case was there any indication that the resistance or susceptibility was influenced by either stock or scion. (Fig. 1.)

In this type of graft both the stock and scion may carry on their own photosynthetic activities. It was thought that if one part of the graft were kept in the dark, so that it would have to depend upon the other part for its photosynthetic products, the results might be different. To test this, either the stock or the scion of the different combinations of several plants were enclosed by improvised boxes of black paper. Inspection showed that the enclosed leaves were devoid of chlorophyll after about 10 days. The boxes were removed and the plants were inoculated in the usual manner and incubated in a dark room. Here again the resistance or susceptibility was in no way modified.

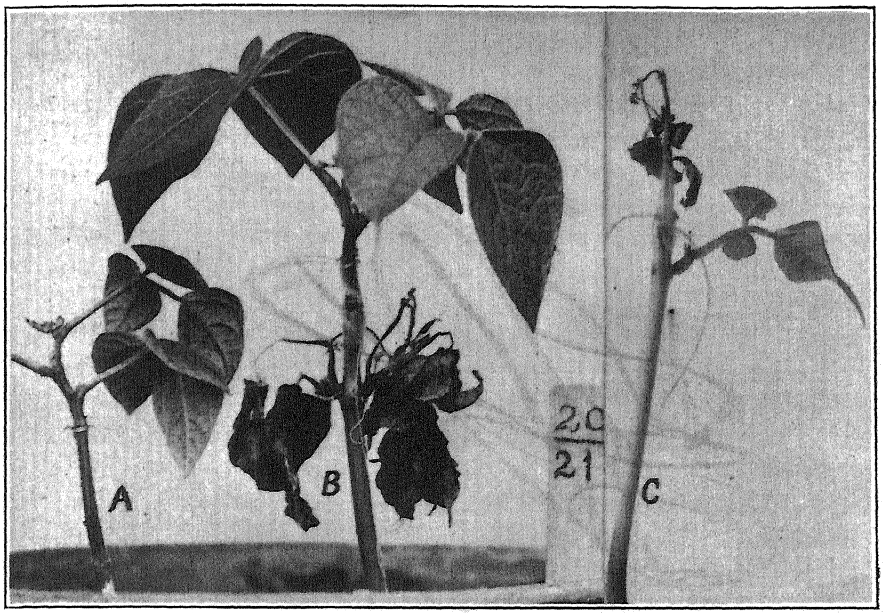


FIG. 1. A, plant of the resistant variety No. 20; B, a grafted plant with a resistant scion and susceptible stock; C, a grafted plant with susceptible scion and resistant stock. All three plants were inoculated with the same physiologic form of *Colletotrichum lindemuthianum*.

These experiments would seem to indicate that, if resistance or susceptibility is due to a specific substance in the plant, this substance is not translocated from scion to stock or *vice versa*, or else it is modified by the cells which receive it.

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PHYTOPATHOLOGICAL NOTES

Polymorphism of Leptosphaeria coniothyrium (Fckl.) Sacc.—Pathologists have doubtless observed on both young and old twigs and stems of *Rosa* sp. slightly reddish, spotted and streaked, pimpled or water-soaked areas. These diseased areas crack and continue to increase in size and a callus forms over the cracks. Later, a definite canker may develop. On the leaf are spots not unlike those caused by Phyllosticta and Cercospora.

When isolations are made from the twig, stem, leaf, hip, and seed, a fungus is obtained which macroscopically resembles a yeast. It consists of budding cells that put out one or more protuberances resembling small ruptures or swellings which gradually thicken and then produce new ones similar to those from which they have arisen. The process continues and gives rise to various kinds of saccharomyces-like colonies. This appears to be the initial phase of *Leptosphaeria coniothyrium* and has been reported as *Polyspora* sp. and *Kabatiella* sp. In this note, this phase of the organism is designated as Pseudosaccharomyces.

A Pseudofumago phase is obtained from the budding of Pseudosaccharomyces. On the stem lesions and leaf spots and in culture, it forms short chains or glomerules composed of globular or ellipsoid cells simple or divided by one or two transverse septa and sometimes also by a longitudinal septum.

Transfers from these phases in culture have yielded a mycelium which has developed pycnidia. The conidia are at first hyaline and might be considered as belonging to Phyllosticta or Phoma; however, at maturity the spores become colored. It is evident that Coniothyrium is the name which should be applied to this phase of the fungus. There is also a wide variation in size of the spores.

Coniothyrium has also developed on the twigs, stems, hips, and seed from which Pseudosaccharomyces and Pseudofumago were isolated when the material was put in a damp chamber and kept warm for from ten to eighteen days.

A recent collection has been made from which all five phases, including Leptosphaeria, were obtained from the same canker. Single-spore isolations of *Leptosphaeria coniothyrium* have produced Coniothyrium in culture.

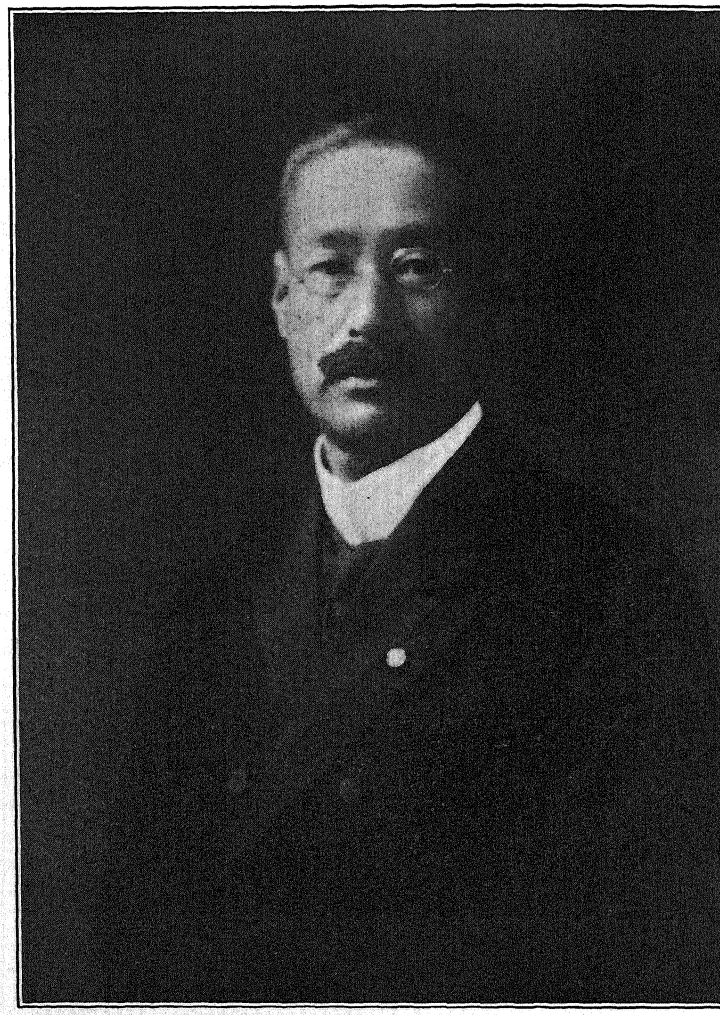
The Pseudosaccharomyces and Pseudofumago phases of the fungus have been isolated from material received from Connecticut, West Virginia, Georgia, Michigan, Indiana, and Kansas, and collected by the writer in New Jersey, District of Columbia, Maryland, and Virginia. It is concluded that *L. coniothyrium* is a polymorphic facultative organism with several phases in its life history.—G. Hamilton Martin, *Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.*

Tobacco Mosaic Control in Mexico.—In September, 1928, Mr. J. O. Menchaca, of Tepic, Mayarat, Mexico, visited the flue cured and Burley tobacco growing sections of the United States and during this trip called on the writer at Lexington, Ky. In discussing tobacco diseases, the writer showed Mr. Menchaca tobacco plants affected with true tobacco mosaic, and other virus diseases of tobacco. He stated that he had had tobacco mosaic pointed out to him by others on the trip, but that he had never seen it in Mexico. In view of the fact that more than 1000 acres of tobacco are grown under his direct supervision each year, the statement probably means that mosaic is extremely rare in his plantings. On questioning him as to the personal habits of the tobacco growers with respect to the use of tobacco, he stated that practically all of them smoked manufactured cigarettes made from Mexican grown tobacco. This tobacco is allowed to heat in bulk, a process which, in itself, is likely to kill the virus if it were present. While we know that many brands of American cigarettes carry the mosaic virus, the chance of spread from cigarettes to tobacco plants is comparatively slight as compared with using viruliferous chewing or smoking tobacco where the hands may readily become contaminated as a result of handling, and breaking or grinding the tobacco in the hands. Thus, even though viruliferous cigarettes were used by the growers during the pulling and setting season, the number of infected plants resulting would be extremely small, and cigarettes made from the resulting tobacco would be expected to be nearly free from infection. As personally used tobacco is apparently the chief source of introduction of mosaic into the field in Kentucky (Ky. Exp. Sta. Bul. 280), it seems fair to assume that such would be the case in other parts of the world, and that mosaic control in Mr. Menchaca's plantings is the direct result of the habit of the growers of using tobacco only in the form of manufactured cigarettes made from tobacco which we have reason to believe is free from mosaic.—W. D. VALLEAU, *University of Kentucky, Lexington, Ky.*

FINANCIAL STATEMENT OF LYMAN MEMORIAL FUND SEPTEMBER 10, 1929

Cash in bank on savings account	$ 412.44
Pledges	670.40
Total	$1,082.84

This is an increase of $367.84 since May 20 when the fund totaled $715. The number of contributors is 73 and the amounts of pledges and donations range from $1.00 to $100.



TŌJI NISHIDA

PHYTOPATHOLOGY

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TŌJI NISHIDA
1874–1927

R. KENT BEATTIE[1]

Dr. Tōji Nishida was born in Tokyo, Japan, on April 15, 1874. He died at Kumamoto on June 19, 1927. He belonged to a distinguished family, his father having been a Court poet and his father-in-law a jurist and member of the Diet.

Dr. Nishida completed the course at the Sapporo Agricultural College (now the Hokkaido Imperial University) in 1899, from which institution he received the degree of Bachelor of Agriculture. He was a pupil of Dr. Kingo Miyabe, known to so many plant pathologists in the United States. His baccalaureate thesis was entitled: "Studies on the Japanese species of Exoascaceae" and his interest in this fungus group was retained throughout life. In 1919 he became a Doctor of Agriculture.

After graduation from the Sapporo Agricultural College, he was at once appointed a member of the staff of the Imperial Agricultural Experiment Station at Nishigahara near Tokyo where he served till 1904 when he was transferred to the Kyushu branch of the Imperial Experiment Station located at Kumamoto. During the Russo-Japanese war he served with the Japanese Army, returning to the Kumamoto Station in 1906.

In 1914 he was promoted to Plant Pathologist and became the Director of the Kobe Substation of the Imperial Plant Quarantine Service. In 1923 he left the Quarantine Service and returned to the Kyushu Branch Experiment Station. With the closing of this station in 1924, Dr. Nishida entered the Kumamoto Medical College as a student with the purpose of acquiring a sufficient knowledge of human diseases to permit him to enter the field of Comparative Plant and Animal Pathology. However, before his course was completed, apoplexy laid him low at the age of fifty-three. He is survived by his wife and two sons.

Dr. Nishida was interested chiefly in cereal and fruit diseases, more especially in citrus diseases and the Exoascaceae. He was the author of at least forty-two papers the titles of which are listed in the appended bibliography.

[1] The data herein have been kindly furnished by Dr. Nishida's eldest son, Mr. Masaaki Nishida.

He was author or original collector of the following species of fungi: *Taphrina alni-incanae* Nishida, *T. alni-japonicae* Nishida, *T. betulicola* Nishida, *T. coryli* Nishida, *T. hiratsukae* Nishida, *T. mume* Nishida, *T. struthiopteris* Nishida, *Gloeosporium foliicolum* Nishida, *Triphragmium nishidanum* Diet., *Puccinia nishidana* P. Henn.

Dr. Nishida's worth to Japan was recognized by the conferring upon him of the "Second Grand Decoration of the fourth rank, and the fourth Order of Merit."

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PATHOGENICITY AND CULTURAL BEHAVIOR OF *USTILAGO ZEAE* (BEKM.) UNG. FROM DIFFERENT LOCALITIES[1]

A. H. EDDINS[2]

INTRODUCTION

In developing high yielding synthetic varieties of corn, it is essential that inbred strains making up such varieties be free of disease if maximum yields are to be obtained. The recent work of Christensen and Stakman (3) on *Ustilago zeae* (Bekm.) Ung. indicates that the corn breeder is now confronted with the question as to whether or not a particular synthetic variety of corn, resistant to the smut of the section where it is developed, will be resistant to that of other sections where this variety may be grown.

The studies reported in this paper were undertaken to determine whether *Ustilago zeae,* as it occurred throughout Iowa, differed in its ability to produce smut on plants of selected inbred lines of corn. This has involved mainly a detailed study of the pathogenicity and cultural behavior of *U. zeae* collected from twelve different localities in the State.

REVIEW OF LITERATURE

Physiologic specialization has been observed in several genera of the smut fungi (11, 31, 32, 33, 42). Melchers (26) indicated the presence of physiologic differences in *U. zeae.* He found that selections and crosses of corn resistant in one locality or State were much more susceptible in other sections. Tisdale and Johnston (40) also found that cultures of *U. zeae* from some sources were much more virulent than cultures of the organism from other sources. Stakman and Christensen (38) obtained evidence of physiologic forms of *U. zeae,* and later these authors (3) came to the conclusion that *U. zeae* is a group species made up of numerous physiologic forms. Finally, Griffiths (14) reported that six different collections of corn smut from the Arlington Experiment Farm showed differences in culture and pathogenicity.

Recent investigations point to the fact that certain fungi behave as if sexual strains were present. Blakeslee (1) found both heterothallism and homothallism in the Mucors. Dodge (9) produced fertile hybrid perithecia in culture by crossing two species of the red bread mold fungi, *Neurospora*

[1] This paper is the major part of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree of Doctor of Philosophy, granted December 21, 1928.

[2] Grateful acknowledgment is made to Drs. J. B. Wentz, E. W. Lindstrom, and J. C. Gilman for criticisms and suggestions; and to Mr. D. G. A. Kelbert for making all photographs.

sitophila (Mont.) Shear and B. O. Dodge and *N. tetrasperma* Shear and B. O. Dodge. Craigie (4, 5), while working with *Puccinia helianthi* Schw. and *P. graminis* Pers., found that sex existed in the rust fungi. Different workers (17, 23, 28, 43) have reported the quality of sex in several species of the Hymenomycetes.

Tisdale, Melchers, and Clemmer (41) advanced the hypothesis that a new form of smut in milo and hegaria sorghums arose through the hybridization of *Sphacelotheca sorghi* (Lk.) Clint. and *S. cruenta* (Kuehn.) Potter by fusion between hyphae of the two species growing in the same host plant. Kniep (22) reported conjugation occurring between germ tubes of different species of Ustilago. Dickinson (6, 7) reported sexual strains in *Ustilago levis* (Kell. and Sw.) Magn. and *U. hordei* (Pers.) Kell. and Sw. He discovered that two of the four isolated sporidia of a single chlamydospore were of one sex and two of another sex. Stakman (37) concluded that *U. zeae* was heterothallic, as the fusion of two multisporidial strains of opposite sex was necessary for the production of chlamydospores in the host plant.

Workers differ in their opinions as to where sexual fusions occur in *U. zeae*. Maire (25), Hitchcock and Norton (16), and Sartoris (34) have observed fused sporidia. Lutman (24) was unable to determine how the multinucleated vegetative mycelium developed into the chlamydospore. Rawitscher (30), Seyfert (36), and Stakman (37) have observed what they thought to be sexual fusions in *U. zeae* growing in the host plant prior to the formation of chlamydospores.

MATERIALS AND METHODS

Materials. Inbred lines of corn were used in these studies, for they differ from each other in smut susceptibility (12, 15, 19, 20, 21). The lines[3] had been grown from three to five years in the experimental plots at Ames and notes on smut infection had been taken on most of them each year. The inbreds, with their parents and smut reaction, are shown in table 1.

The twelve collections of smut used in this work obtained from the sources indicated in table 2 and the accompanying map, figure 1. In addition to these collections, five cultures isolated from one smut gall were used and designated A1, A2, A3, A4, and A5. Emery R. Ranker, of the U. S. Dept. of Agr., Washington, D. C., furnished six monosporidial cultures known as Wisconsin 1, Arlington Farm 2, Michigan 10, Arlington Farm 14, South Dakota 37, and Kansas 38.

Methods. Pure cultures of *U. zeae* were obtained in two ways: (1) Chlamydospores were soaked in a 1 per cent solution of copper sulphate

[3] These were obtained from E. W. Lindstrom and Merl T. Jenkins, of the Iowa Agricultural Experiment Station, Ames, Iowa.

TABLE 1.—*Data showing smut reaction of inbred lines of corn used in the smut infection studies*

Variety	Inbred line number	Number years selfed	Percentage smut				
			1923	1924	1925	1926	1927
Four County White	5	5	—[a]	10	60	10	30
Four County White	35	5	—	20	40	30	20
Four County White	97	5	0	0	0	0	20
C. I. No. 133	134	5	10	30	50	10	65
Iodent	195	4	0	0	0	0	7
Iodent	224	5	—	20	30	10	50
Iodent	224[a]	5	—	20	60	10	30
Iodent	238	5	0	0	0	0	0
C. I. No. 204	273	5	—	10	40	—	50
Lancaster Sure Crop	311	4	0	0	0	—	0
Black's Yellow Dent	344	5	0	0	0	0	55
Black's Yellow Dent	356	5	—	20	40	20	64
Reid's Yellow Dent, Proudfit's strain	358	5	—	10	50	20	50
Krizer Bros. Yellow Dent	379	5	—	20	50	0	30
Osterland Yellow Dent	420	4	0	0	0	—	21
Clark's Yellow Dent	440	5	0	0	0	0	0
Clark's Yellow Dent	440[a]	5	0	0	0	0	7
Walden's Yellow Dent	452	5	10	30	60	25	50
Walden's Yellow Dent	460	5	0	0	0	0	0
Evergreen Sweet	S314	3	0	0	0	0	5
Golden Bantam Sweet	6883	3	0	0	0	0	10

[a] No notes taken on smut infection.

TABLE 2.—*Sources of twelve collections of Ustilago zeae in Iowa*

Collection	Locality	Collector
1	George, Lyon Co.	James C. Halasey
2	Buffalo Center, Winnebago Co.	Fred G. Bliss
3	Postville, Allamakee Co.	Fred C. O'Riley
4	Cherokee, Cherokee Co.	Lester M. Blanch
5	Goldfield, Wright Co.	Miller S. Nelson
6	Manchester, Delaware Co.	Earl New
7	Castana, Monona Co.	Carl D. Kiser
8	Ames, Story Co.	A. H. Eddins
9	Center Junction, Jones Co.	Paul N. Smith
10	Elliott, Montgomery Co.	J. H. Petty
11	Lamoni, Decatur Co.	F. S. Parks
12	Columbus Junction, Louisa Co.	Robert Duncan

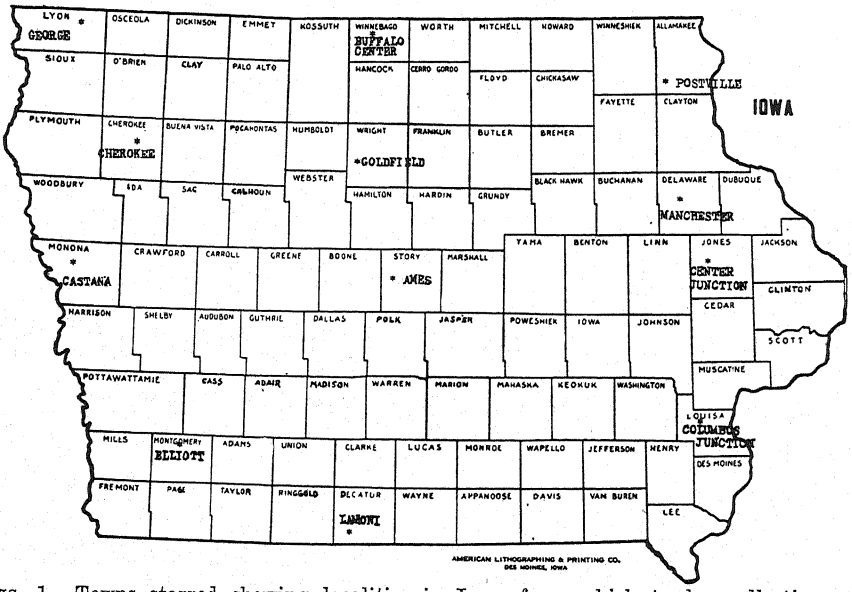


FIG. 1. Towns starred showing localities in Iowa from which twelve collections of *Ustilago zeae* were made.

from 12 to 60 hours, and a pure culture was developed by removing a little of the spore suspension with a sterile platinum loop and streaking it across a carrot agar slant in a test-tube (3). (2) Pure cultures of the fungus were

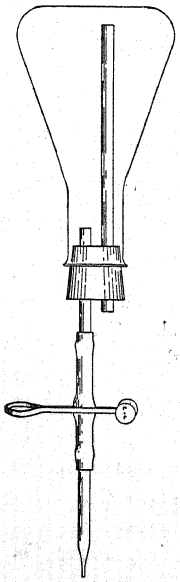


FIG. 2. Erlenmeyer flask used in dropping inoculum on apical buds.

isolated from the interior of fresh unbroken smut galls. The fungus was grown on a 3 per cent carrot agar, and conidial suspensions for inoculation were prepared by growing the conidia in carrot decoction in Erlenmeyer flasks from six to ten days.

The plants were inoculated by two methods: (1) Conidial suspensions of *U. zeae* were injected into young plants with a hypodermic syringe when the plants were in the three- to four-leaf stage (40). (2) Conidial suspensions were dropped on the apical buds of plants which averaged a foot in height (2, 14, 16, 40). A pipette was used to drop inoculum into the tops of plants grown in the greenhouse, and an Erlenmeyer flask fitted with clamp, rubber, and glass tubes, as is shown in figure 2, was used in field inoculations.

GREENHOUSE EXPERIMENTS

Inoculation experiments were conducted in the greenhouse in the winter of 1927–28, at Ames, Iowa. Inbred lines which had proven to be susceptible, moderately susceptible, and resistant to smut under natural field conditions at Ames were used in these experiments. The corn was grown in greenhouse flats and in flower pots. Plants grown in flats were inoculated hypodermically, and those in pots by dropping the inoculum on the apical buds. The greenhouse, kept moist by a thorough spraying twice daily, was maintained at a mean temperature of 78° F.

Pathogenicity of cultures of U. zeae from twelve different localities in Iowa. The results obtained from inoculating ten inbred lines of corn by the hypodermic and dropper methods are given in tables 3 and 4, respectively. The percentage of plants infected includes those with galls and those that developed chlorotic and necrotic areas on the leaves, leaf sheaths, and stalks of the young seedlings. The data, presented in these two tables and graphically shown in figure 3, show that the twelve cultures differed from each other in the percentage of plants infected and the percentage of plants with galls in each of the ten inbred lines of inoculated corn. The check plants were inoculated with sterile carrot decoction and none of them developed smut galls or became chlorotic or necrotic.

A summary of the results of the inoculations is given in table 5. When the ten inbreds were inoculated hypodermically, cultures 3, 6, 8, 9, and 12 each produced galls on from seven to nine lines of corn. Cultures 1, 2, 4, 10, and 11 are in a group which formed galls on from two to four inbreds out of the total of ten inoculated. Culture 5 produced galls on one line of corn and culture 7 failed to form galls by the hypodermic method of inoculation.

When the inoculum was dropped on the apical buds of plants, the results differed from those obtained when the same inbreds were inoculated hypodermically with the same smut cultures. The data in table 5 show that cultures 8 and 6 formed galls on seven and eight lines, respectively. Galls were

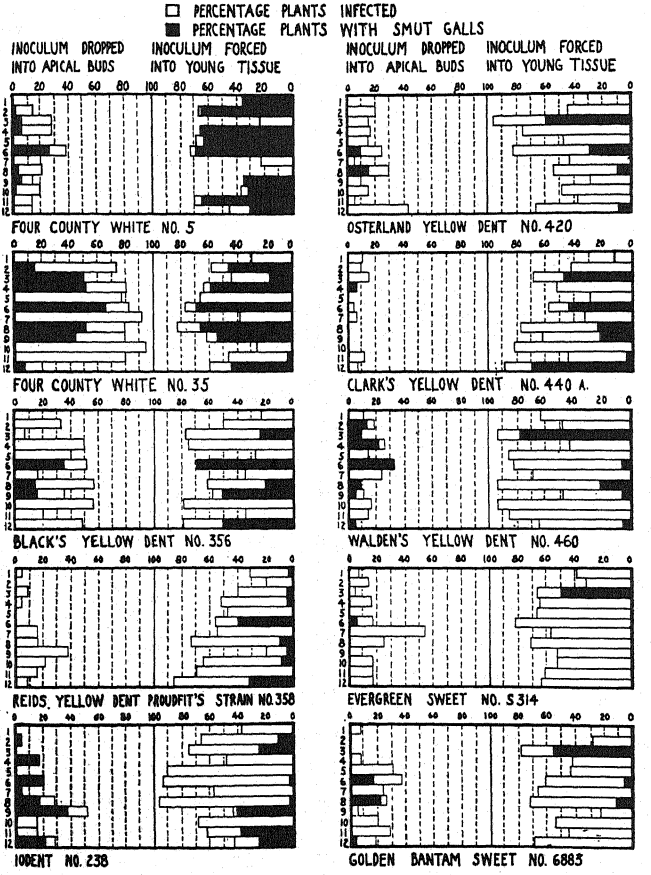


FIG. 3. Results of inoculating ten inbred lines of corn with twelve cultures of *Ustilago zeae*. Cultures of *U. zeae* Nos. 1 to 12 appear on left and percentage smut at the top of each histograph.

produced on three to five inbreds when inoculated with cultures 2, 3, 4, 9, and 12. Cultures 7 and 10 each produced galls on only one line and no galls were formed when cultures 1, 5, and 11 were used.

The inbred lines were used as differential hosts (3, 40) and the results show that there is a difference in the pathogenicity of the cultures of *U. zeae* from different localities in Iowa, for several cultures produced galls on only two or three lines or not at all, while other cultures formed galls on from seven to nine inbreds.

Pathogenicity of five cultures of U. zeae isolated from one smut gall. The results of inoculating six inbreds with five cultures of *U. zeae* from the same smut gall are reported in tables 6 and 7 and graphically shown in

TABLE 3.—*Results of inoculating ten inbred lines of corn with twelve cultures of Ustilago zeae by forcing the inoculum into the plants with a hypodermic syringe*

	Four County White No. 5			Four County White No. 35			Black's Yellow Dent No. 356			Reid's Yellow Dent Proudfit's Strain No. 358			Iodent No. 238			Osterland Yellow Dent No. 420			Clark's Yellow Dent No. 440a			Walden's Yellow Dent No. 460			Evergreen Sweet No. S314			Golden Bantam Sweet No. 6883		
	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
	31	36	36	24	29	0	13	23	0	29	31	4	25	36	0	25	0	0	28	12	0	27	63	0	25	39	0	16	0	0
	34	67	65	26	58	46	20	50	0	24	20	0	25	68	12	16	44	0	27	41	0	27	48	0	25	32	0	21	29	0
	35	23	0	25	44	16	25	77	24	22	40	5	20	75	25	22	95	59	28	68	47	28	93	78	24	66	50	18	78	55
	33	66	66	22	64	59	16	75	0	21	52	5	23	48	0	16	75	0	27	52	0	28	43	0	22	64	0	21	43	0
	35	69	63	24	66	0	26	27	0	19	47	0	16	90	0	17	47	0	27	29	0	28	85	0	27	66	0	18	44	0
	32	72	69	13	77	69	26	69	69	23	56	40	27	93	4	17	82	29	28	57	43	28	82	7	22	82	0	15	60	6
	37	22	0	21	38	0	26	35	0	22	55	0	21	57	0	24	42	0	28	32	0	27	69	0	28	57	0	18	66	0
3	35	0	0	12	83	66	24	62	20	19	74	10	23	96	4	22	54	9	26	77	23	27	93	22	24	71	0	18	72	11
)	31	35	35	24	62	54	26	58	50	20	20	5	23	43	40	17	29	0	28	61	21	27	48	7	27	52	0	22	23	0
)	35	37	31	23	26	0	24	79	0	23	65	9	22	68	0	23	48	0	26	81	0	27	93	0	27	52	0	24	54	0
1	37	70	65	24	46	4	23	35	0	28	69	0	21	62	38	19	37	0	27	44	4	28	85	0	25	60	0	22	45	0
2	40	45	30	25	60	44	24	79	50	22	86	32	24	42	25	21	66	9	26	88	69	28	64	7	24	62	0	26	69	0
eck	40	0	0	18	0	0	26	0	0	19	0	0	23	0	0	18	0	0	26	0	0	28	0	0	27	0	0	19	0	0

TABLE 4.—*Results of inoculating ten inbred lines of corn with twelve cultures of Ustilago zeae by dropping the inoculum on the apical buds of the plants*

Four County White No. 5			Four County White No. 35			Black's Yellow Dent No. 356			Reid's Yellow Dent Proudfit's Strain No. 358			Iodent No. 238			Osterland Yellow Dent No. 420			Clark's Yellow Dent No. 440a			Walden's Yellow Dent No. 460			Evergreen Sweet No. S314			Golden Bantam Sweet No. 6883		
Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
47	0	0	26	0	0	14	0	0	23	4	0	17	0	0	12	0	0	25	11	0	18	11	0	27	7	0	27	8	0
50	14	2	23	74	15	21	33	0	22	0	0	23	4	4	20	20	0	28	10	0	21	19	14	28	14	0	24	0	0
48	28	6	22	50	50	13	7	0	23	9	0	21	0	0	20	15	0	27	15	0	20	10	10	23	4	0	27	0	0
46	28	6	27	81	52	24	50	0	21	4	0	23	17	17	18	17	0	27	7	7	23	26	22	25	16	0	25	8	0
49	0	0	26	77	0	21	40	0	25	0	0	23	0	0	20	15	0	28	0	0	21	14	0	22	0	0	26	31	0
50	38	26	24	83	66	23	52	35	19	0	0	25	20	20	20	25	10	27	4	0	24	33	33	24	17	5	24	37	17
49	20	0	25	92	0	18	16	0	24	16	0	25	4	4	19	5	0	28	7	0	21	24	0	26	54	0	21	24	0
48	21	4	25	80	52	21	57	14	18	16	0	24	29	17	20	30	15	28	0	0	22	9	9	24	25	0	23	26	22
50	14	6	25	80	44	20	35	15	26	38	0	21	52	38	20	10	0	28	0	0	19	11	5	26	0	0	22	5	0
46	19	2	22	95	0	16	56	0	27	22	0	20	15	0	20	15	0	26	0	0	19	16	0	23	17	0	25	20	0
45	13	0	25	80	0	25	20	0	26	15	0	20	15	0	21	5	0	25	12	0	21	14	0	28	17	0	24	29	0
46	13	0	15	20	7	21	48	0	22	9	0	24	29	21	21	43	0	25	8	0	20	5	5	24	17	0	26	19	4
46	0	0	27	0	0	23	0	0	25	0	0	22	0	0	15	0	0	24	0	0	21	0	0	25	0	0	26	0	0

TABLE 5.—*Number of lines of corn showing necrosis, chlorosis, and galls when inoculated with twelve smut cultures hypodermically and by dropping the inoculum on the apical buds*

Method of inoculation	Number of lines inoculated	Number lines infected when inoculated with											
		No. 1		No. 2		No. 3		No. 4		No. 5		No. 6	
		Nec.[a] Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present
Inoculum forced into young tissue hypodermically	10	8	2	10	3	10	9	10	3	10	1	10	9
Inoculum dropped into apical buds	10	5	0	8	4	8	3	10	5	5	0	9	8

Method of inoculation	Number of lines inoculated	Number lines infected when inoculated with											
		No. 7		No. 8		No. 9		No. 10		No. 11		No. 12	
		Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present
Inoculum forced into young tissue hypodermically	10	10	0	9	8	10	7	10	2	10	4	10	8
Inoculum dropped into apical buds	10	10	1	9	7	8	5	9	1	10	0	10	4

[a] Nec., necrotic; Clo., chlorotic.

figure 4. In a few cases, a culture of smut produced galls on plants of an inbred when inoculated by one method, but failed to form galls on the same inbred when inoculated by the other. Plants infected with smut when inoculated by the dropper method are shown in figure 5. The five cultures from the same smut gall differed from each other in the percentage of plants infected and the percentage with galls when the six inbreds were inoculated.

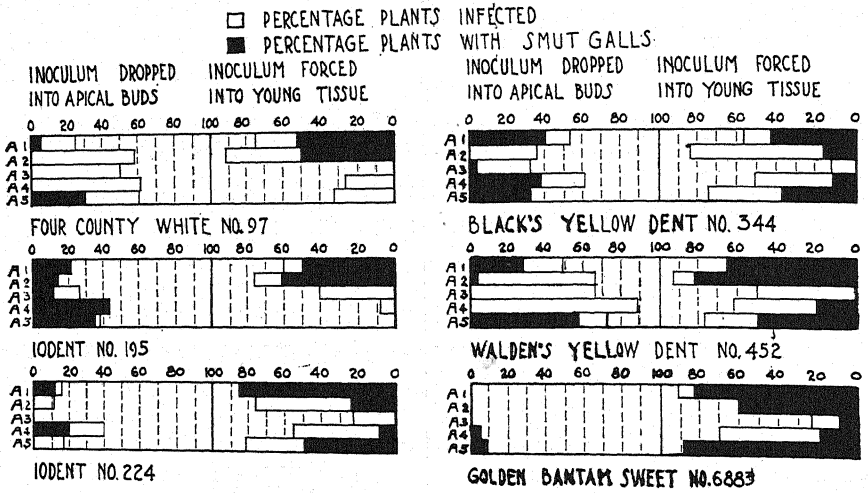


FIG. 4. Results of inoculating six inbred lines of corn with five cultures of *U. zeae* from the same smut gall. Cultures A1 to A5 appear on left and percentage smut at top of each histograph.

The data in table 8 are a summary of the results of the inoculations. When the seedlings were inoculated hypodermically, cultures A1 and A2 produced galls on all six lines. Cultures A4 and A5 each formed galls on four inbreds and A3 produced galls on only one line. When the inoculum was dropped into the tops of the plants, A1 and A5 each produced galls on five lines. A4 formed galls on four lines and A2 and A3 each produced galls on only two inbreds. The cultures from the same smut gall differed from each other in their ability to produce smut about as much as did the twelve cultures isolated from galls collected in different localities in Iowa. The results not only indicate that *U. zeae* in the same locality (3, 14) varies in its pathogenicity, but they also show that a single smut gall may be composed of a great number of pathogenic variants of the organism, as indicated by Griffiths (14).

Pathogenicity of monosporidial cultures and their combinations. Six single-sporidial cultures of *U. zeae* were obtained from Emery R. Ranker, Washington, D. C. Seedlings of five inbred lines of corn were inoculated hypodermically with these monosporidial cultures and their paired combina-

tions. The combinations were made at the time of inoculation by mixing equal quantities of the inoculum of each monosporidial culture of which the combination consisted. The results of the inoculations are given in table 9. One monosporidial culture, South Dakota 37, produced chlamydospores in host tissue, and the other five cultures did not. The results of this pathogenicity test led to the question as to whether *U. zeae* was heterothallic or homothallic or both. The production of chlamydospores in corn plants inoculated with paired monosporidial cultures and the absence of chlamydospores in plants inoculated with such cultures alone served as the criterion for

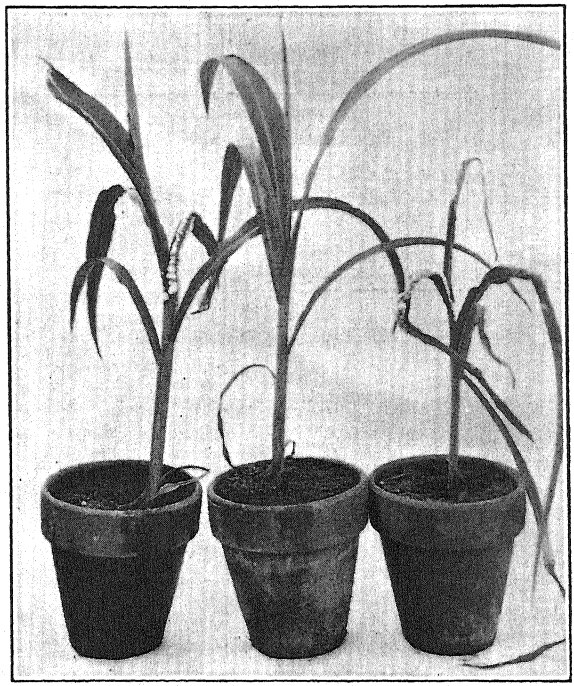


FIG. 5. Two outer plants infected with smut and center plant not infected when inoculated by the dropper method in the greenhouse.

heterothallism (37). A monosporidial culture was considered homothallic if it alone produced chlamydospores in the host plant.

Previous workers (17, 28) have found that cultures of certain Hymenomycetes, which are regularly heterothallic, changed spontaneously to the homothallic condition. The fact that only one of the six monosporidial cultures of *U. zeae* produced chlamydospores in plants inoculated with the cultures alone suggests that this organism also is regularly heterothallic and that those monosporidial cultures which produce chlamydospores in host

TABLE 6.—*Results of inoculating six inbred lines of corn with five cultures from the same smut gall by injecting the inoculum into the plants with a hypodermic syringe*

Smut culture	Four County White No. 97			Iodent No. 195			Iodent No. 224			Black's Yellow Dent No. 344			Walden's Yellow Dent No. 452			Golden Bantam Sweet No. 6883		
	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
A1	12	75	53	10	60	50	20	85	86	26	57	43	17	70	65	24	92	83
A2	14	92	50	13	77	61	20	76	24	25	84	16	18	94	83	25	60	60
A3	15	0	0	10	40	0	22	23	0	25	12	0	18	50	0	26	23	8
A4	15	27	0	13	8	0	22	55	9	26	46	11	21	62	19	27	70	18
A5	15	33	0	7	0	0	22	82	50	27	74	37	22	77	50	28	89	89
Check ...	15	0	0	7	0	0	24	0	0	28	0	0	21	0	0	27	0	0

TABLE 7.—*Results of inoculating six inbred lines of corn with five cultures from the same smut gall by dropping the inoculum into the tops of the plants*

Smut culture	Four County White No. 97			Iodent No. 195			Iodent No. 224			Black's Yellow Dent No. 344			Walden's Yellow Dent No. 452			Golden Bantam Sweet No. 6883		
	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls	Number plants inoculated	Per cent plants infected	Per cent plants with galls
A1	20	25	5	23	22	22	24	16	13	17	53	41	21	48	28	15	0	0
A2	17	58	0	21	14	14	25	12	0	22	36	0	27	66	4	23	0	0
A3	12	50	0	19	26	12	16	0	0	24	33	4	24	66	0	19	0	0
A4	16	62	0	25	44	44	25	40	20	21	62	38	17	89	0	22	5	5
A5	13	61	30	26	38	35	24	17	0	18	33	33	26	73	57	22	9	9
Check ..	20	0	0	17	0	0	25	0	0	23	0	0	22	0	0	21	0	0

TABLE 8.—*Summary showing lines of corn infected with smut when hypodermically inoculated with five cultures of U. zeae from the same smut gall, and by dropping the inoculum into the tops of the plants*

Method of inoculation	Number of lines inoculated	Number lines infected when inoculated with									
		A1		A2		A3		A4		A5	
		Nec.[a] Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present	Nec. Clo.	Galls present
Inoculum forced into young tissue hypodermically	6	6	6	6	6	5	1	6	4	5	4
Inoculum dropped on apical buds	6	5	5	5	2	4	2	6	4	6	5

[a] Nec., necrotic; Clo., chlorotic.

TABLE 9.—*Results of inoculating five inbred lines of corn hypodermically with monosporidial cultures of Ustilago zeae and with different combinations of these cultures*

Smut culture[a]	Iodent No. 224a			Krizer Bros. Yellow Dent No. 379			C. I. No. 204 (273)			Lancaster Sure Crop No. 311			Clark's Yellow Dent No. 440a		
	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls
Wis. 1	30	10	0	19	17	0	28	6	0	5	0	0	10	5	0
Arl. Farm 2	26	7	0	22	7	0	34	5	0	7	0	0	9	7	0
Mich. 10	29	19	0	12	11	0	30	11	0	6	1	0	11	3	0
Arl. Farm 14	30	4	0	17	10	0	36	3	0	6	0	0	10	6	0
S. Dak. 37	7	3	3	—	—	—	—	—	—	5	2	1	10	7	3
Kan. 38	28	13	0	21	12	0	32	5	0	7	5	0	10	6	0
Wis. 1 + Arl. Farm 2	32	4	0	22	10	0	34	6	0	1	0	0	10	5	0
Wis. 1 + Mich. 10	27	23	7	11	10	10	34	9	0	2	1	0	9	6	0
Wis. 1 + Arl. Farm 14	30	6	0	15	0	0	33	0	0	5	3	0	9	3	0
Wis. 1 + S. Dak. 37	9	7	4	—	—	—	—	—	—	3	3	0	10	3	3
Wis. 1 + Kan. 38	32	25	17	15	14	14	26	13	6	2	0	0	9	7	5
Arl. Farm 2 + Mich. 10	31	24	6	17	17	11	33	21	4	8	6	0	10	5	3
Arl. Farm 2 + Arl. Farm 14	28	27	6	13	12	2	27	9	0	5	5	0	10	7	0
Arl. Farm 2 + S. Dak. 37	7	6	6	—	—	—	—	—	—	5	5	0	8	7	5
Arl. Farm 2 + Kan. 38	25	19	6	15	14	8	28	15	1	6	2	2	10	8	6
Mich. 10 + Arl. Farm 14	31	23	0	24	0	0	30	1	0	4	0	0	9	2	0
Mich. 10 + S. Dak. 37	6	5	5	—	—	—	—	—	—	5	2	2	9	8	4
Mich. 10 + Kan. 38	27	8	0	15	0	0	24	2	0	4	0	0	10	7	0
Arl. Farm 14 + S. Dak. 37	10	7	5	—	—	—	—	—	—	5	2	2	10	8	3
Arl. Farm 14 + Kan. 38	32	5	0	13	0	0	26	1	0	4	3	0	10	6	0
S. Dak. 37 + Kan. 38	8	5	5	—	—	—	—	—	—	5	4	4	10	7	4
Check	30	0	0	23	0	0	28	0	0	6	0	0	10	0	0

[a] Wis., Wisconsin; Arl. Farm, Arlington Farm; Mich., Michigan; S. Dak., South Dakota; Kan., Kansas.

tissue are homothallic and arise through mutation. However, Maire's (25) studies on the nuclear behavior of *U. zeae* suggests that homothallic cultures may arise in another manner. Maire found that when a chlamydospore of *U. zeae* germinated, its nucleus passed into the promycelium and divided. Some times the daughter nuclei did not divide, at other times only one divided, while, normally, both daughter nuclei divided. Upon germination chlamydospores formed promycelia consisting either of two, three, or four cells, each cell of which contained one nucleus. The nucleus of each cell divided when a sporidium was budded off and one of the daughter nuclei passed into this sporidium. If it is assumed that the first division of the nucleus in the promycelium is equational and the second reductional, sporidia budded off from a promycelial cell whose nucleus has undergone only one division might possess a nucleus in the diploid condition. Hanna (18) stated that segregation of factors may occur at both divisions of the spore nucleus. A sporidium budded off from a promycelial cell whose nucleus has undergone two divisions, before the final one which produces a daughter nucleus that passes into the sporidium, would possess a nucleus in the haploid condition. Thus it is possible that monosporidial cultures of *U. zeae,* such as South Dakota 37, that produce chlamydospores in host tissue, are homothallic, and are in a diploid condition if they originate from sporidia borne on a promycelial cell with an unreduced nucleus. Such diploid sporidia, theoretically, could be borne only by two or three-cell promycelia. Monosporidial cultures which do not produce chlamydospores in host tissue must be in a haploid condition and originate from sporidia borne on cells of promycelia whose nuclei have undergone reduction division. The data in table 9 show that certain monosporidial cultures have to be paired to produce chlamydospores in corn plants (10, 18).

TABLE 10.—*All possible pairings of five monosporidial cultures of Ustilago zeae*

		A[a]	B	C		D
		Wis. 1	Arl. Farm	Mich. 10	Kan. 38	Arl. Farm 14
A	Wis. 1	–[b]	–	+	+	–
B	Arl. Farm 2	–	–	+	+	+
C	Mich. 10	+	+	–	–	–
	Kan. 38	+	+	–	–	–
D	Arl. Farm 14	–	+	–	–	–

[a] A, B, C, and D sexual strains.

[b] – No chlamydospores formed in host tissue; + chlamydospores formed.

When the five monosporidial cultures were paired, as shown in table 10, mating occurred between certain pairs which produced chlamydospores in host tissue. This indicates the presence of sex strains in *U. zeae*. It has been demonstrated that such strains occur in several genera of the Basidiomycetes, including the genus Ustilago. Theories based on the presence of two and more than two sex strains have been used to explain the sexual reactions of these fungi.

The bisexuality theory explained the sexual behavior of *Aleurodiscus polygonius* (Pers.) Hoehnel and Litsch (23), *Coprinus rostrupianus* Hansen (28) and *C. radians* (Desm.) Fr. (43). The same theory failed to explain the sexual nature of *C. lagopus* Fr. and *C. niveus* (Pers.) Fr. (27), and Hanna (17) proved that four sex strains were present in *C. lagopus*. Sass (35) has shown that the presence of one pair of Mendelian factors does not determine the sex of paired mycelia of *C. ephemerus* (Bull.) Fr.

Kniep (22) noted sexual reactions among conjugating germ tubes of several different species of Ustilago. Dickinson (7, 8) found that there were only two sexes in *Ustilago levis* and two in *U. hordei,* and Stakman (37) concluded from his work with paired multisporidial cultures of *U. zeae* that the presence of two sexes in this fungus explained his results.

The results obtained from mating the five monosporidial cultures of *U. zeae,* referred to in table 10, indicated that the cultures belonged to four sexual groups or strains (10). Michigan 10 and Kansas 38 behaved as if they belonged to the same sex strain, and the other cultures, Wisconsin 1, Arlington Farm 2, and Arlington Farm 14, behaved as if they belonged to each of three other sex strains. The four sex strains to which the five cultures belonged were designated A, B, C and D. Chlamydospores were produced in host tissue when A was paired with C but not when paired with B and D. Chlamydospores also were produced when B was paired with C and D. No chlamydospores were formed when C was paired with D.

Pathogenicity of multisporidial cultures and their combinations. A test similar to the preceding one with monosporidial cultures was made to study the behavior of three multisporidial cultures and their different combinations. Iowa cultures 1, 5, and 7 were selected for this study, for, as indicated by their behavior, they had apparently become weakly pathogenic (Table 3). The data in table 11 show that multisporidial cultures 1, 5, and 7 produced no galls. Their behavior was similar to that of the five monosporidial cultures reported in the preceding test. However, cultures 1 and 5 had previously formed galls on plants in the greenhouse (Table 3). Culture 7 had produced galls on one inbred line (Table 4). In table 11 it is shown that culture 1 + 7 and 5 + 7 produced galls and 1 + 5 did not. The interaction of the proper sex factors may have been responsible for the production of galls by the first two combinations, and the inability of the

TABLE 11.—*Virulence of Iowa cultures 1, 5, and 7 and their combinations on four inbred lines of corn inoculated hypodermically*

Smut culture	Four County White No. 5			Iodent No. 224a			Iodent No. 238			Walden's Yellow Dent No. 460		
	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls	Number plants inoculated	Number plants infected	Number plants with galls
1	9	0	0	31	0	0	26	24	0	19	0	0
5	10	0	0	31	7	0	27	23	0	19	3	0
7	8	0	0	29	20	0	28	27	0	16	8	0
1+5	9	0	0	27	7	0	28	22	0	16	1	0
1+7	9	7	4	28	12	0	26	23	16	14	1	0
5+7	9	6	5	30	14	4	25	19	11	19	3	1
Check ...	3	0	0	60	0	0	28	0	0	3	0	0

third combination to form galls may have been due either to the absence of some essential factor or factors or to the presence of lethal agents.

Effect of repeated transfers on the virulence of cultures of U. zeae. Three inbred lines of corn were inoculated hypodermically with four pairs of multisporidial cultures of *U. zeae* from Iowa. Each pair came from the same stock culture. One member of the pair had been transferred only once from the date of its isolation and the other six times. The object of the test was to determine whether repeated transfers to carrot agar, as compared to one transfer, would affect the virulence of the cultures.

Iowa 1, 4, and 6, transferred once, produced galls, as is shown in table 12. Iowa 7 transferred once, did not form galls. None of the cultures produced galls after they had been transferred six times. Loss of virulence has been reported as occurring, also, in pure cultures of *Fusarium conglutinans* Woll. (13). Since the four pairs of cultures of *Ustilago zeae* were of multisporidial origin, it is probable that when isolated, each contained sporidia of at least two sexual groups. When injected into corn plants shortly after isolation, galls were formed by all cultures except Iowa 7. At the end of six transfers of cultures 1, 4, and 6, one or more of the essential sexual groups might have been lost, and the cultures no longer produced galls on the inoculated plants. In the case of Iowa 7 the essential sexual factors probably were lost at the first transfer.

Virulence of conidia of U. zeae exposed to winter temperature. A test-tube culture of *U. zeae* on carrot agar was put in a loosely covered Mason fruit jar and placed in an exposed place on the north side of the greenhouse.

BLE 12.—*Comparison of the virulence of four Iowa cultures transferred only once and transferred six times during a six months' period*

Inbred line of corn	Iowa 1				Iowa 4				Iowa 6				Iowa 7			
	Transferred				Transferred				Transferred				Transferred			
	One time		Six times		One time		Six times		One time		Six times		One time		Six times	
	Number plants inoculated	Number plants infected	Number plants inoculated	Number plants with galls	Number plants inoculated	Number plants infected	Number plants inoculated	Number plants with galls	Number plants inoculated	Number plants infected	Number plants inoculated	Number plants with galls	Number plants inoculated	Number plants infected	Number plants inoculated	Number plants with galls
dent No. 238	27	23	18	26	14	0	27	19	4	26	24	0	27	24	8	28	21	0	28	0	0	27	22	0
id's Yellow Dent Proudfit's Strain No. 358	22	18	18	19	7	0	21	2	0	21	4	0	21	16	12	19	12	0	20	19	0	21	11	0
ark's Yellow Dent No. 440a	19	6	3	21	0	0	21	16	1	21	9	0	20	3	3	23	7	0	23	13	0	24	4	0

Another culture was placed in the refrigerator where stock cultures of various fungi were kept. The cultures used in this test were transferred on the same date from Iowa stock culture No. 8, a pure culture of smut isolated from a gall collected from a field at the Agronomy Farm at Ames. The test was started December 2, 1927, and on April 11, 1928, 131 days later, conidia from both cultures were transferred to sterile carrot decoction. Six days later, when the inoculum was ready, an equal number of plants from one inbred line of corn was inoculated with the two cultures.

The results reported in table 13 show that conidia growing on carrot

TABLE 13.—*Virulence of conidia of U. zeae kept in a refrigerator and exposed to outside winter temperature*

Inbred line of corn	Black's Yellow Dent No. 356	
Treatment of cultures	Culture kept outside	Culture kept in refrigerator
Number of plants inoculated	46	48
Number of plants infected	36	32
Number of plants with galls	25	7

agar, when exposed to outside winter temperature retain their power to infect corn seedlings. In fact, the culture kept outside appeared to be the more virulent of the two, as it produced galls on over three times as many plants as did the culture kept in the refrigerator. These results are comparable to those of Piemeisel (29) who found that sporidia were injured very little by freezing.

Comparative effectiveness of two methods of seedling inoculation. Inbred lines of corn were inoculated by dropping the inoculum on the apical buds of plants and by hypodermic injection of the inoculum into the young tissue. The relative effectiveness of the two methods of inoculation is illustrated in figures 3 and 4 in which are shown the results of inoculating inbred lines with different cultures of *U. zeae*.

It is quite evident that the hypodermic method of inoculation was the more effective in inducing smut, for, with only few exceptions, a higher percentage was obtained by this method than by dropping the inoculum on the buds of the plants. Conidia have to be in contact with susceptible host tissue for infection to occur. The conidia were placed nearer susceptible tissue when the inoculum was hypodermically injected than when dropped on the apical bud.

Relation of the condition of the apical buds to smut infection. Conidial suspensions were dropped into the tops of plants averaging about twelve

inches in height.[4] Apical buds were described as open, two-thirds open, one-third open, and closed, respectively, depending upon the size of the receptacle formed by the leaves at the top of the plant.

The results reported in table 14 show approximately twice as great a

TABLE 14.—*Relation of the condition of the apical buds of plants and percentage of plants smutted when seedlings were inoculated by the dropper method with conidial suspensions of U. zeae*

Condition of apical buds	Total number plants inoculated	Number of plants infected	Per cent plants infected	Number plants with galls	Per cent plants with galls
Open	568	222	39.0	88	15.5
Two-thirds open	2016	445	22.0	132	6.5
One-third open	698	85	12.2	14	2.0
Closed	136	12	8.8	1	.7

percentage of infected plants and plants with galls among the open plants as among those that were two-thirds open, and there was correspondingly less smut on plants with buds one-third open and buds closed. Apparently, open apical buds hold the inoculum and allow conidial suspensions to seep down in the plants and come in contact with young, actively growing and susceptible tissue (2). Inoculum seldom reaches susceptible tissue in plants with closed buds.

FIELD EXPERIMENTS

Field tests of the comparative pathogenicity of certain smut cultures were made during the summer of 1928 at the Florida Agricultural Experiment Station, Gainesville, Florida. The corn for these tests was planted on May 19 and was inoculated at each of two different stages of its growth with inoculum from cultures of *U. zeae* that had proved pathogenic on seedlings in the greenhouse.

The plants inoculated hypodermically the first time were in the three- to four-leaf stage and from five to six inches tall. The inoculum was forced in until it ran out at the tops of the plants. At the time of making the second inoculations the plants ranged in height from one to two and a half feet. An attempt was made to insert the hypodermic needle in the stalk so as to hit the growing point, and 2 cc. of inoculum were forced into each plant.

[4] G. A. Platz, a former graduate student at Iowa State College, has a paper in press dealing with investigations on the structure of the corn plant in relation to its susceptibility to smut.

On examination the growing point was found to be located one to three inches above the soil in typical stalks.

Plants inoculated the first time by dropping the inoculum on the apical buds averaged about a foot in height. About 2 cc. of inoculum were dropped into each plant. The second inoculations by this method were made when the plants were from two to three and a half feet tall. Only a few plants had their tassels showing. In most cases, the tip of the developing tassel was several inches down in the stalk. Notes were taken on infection about two weeks from date of each inoculation, and three to four weeks intervened between the inoculations.

Plants under field conditions became chlorotic and necrotic when inoculated with sterile carrot decoction as well as those inoculated with the conidial suspensions of *U. zeae*. Even before any inoculations were made, a type of chlorosis and necrosis appeared on the leaves of plants scattered throughout the field. Undoubtedly, much of the chlorosis and necrosis of the tissues of inoculated plants was due to smut infection, for, in the greenhouse, only those plants inoculated with corn-smut conidia became chlorotic and necrotic. However, the uncertainty of being able to distinguish between chlorosis and necrosis caused by *U. zeae* and that due to other causes made it necessary, in the field, to use only the appearance of galls on the plants as a true symptom of smut infection.

Pathogenicity of three cultures of U. zeae from one smut gall. The results reported in table 15 show that no smut galls developed on plants of any of the lines hypodermically inoculated on June 2 with conidial suspensions of multisporidial cultures A1, A2, and A3 derived from the same smut gall. These results differ considerably from those obtained in the greenhouse where plants, at the same stage of growth, developed galls when inoculated with conidia from the above cultures. However, it should be noted that only two of the inbreds, Walden's Yellow Dent No. 452 and Golden Bantam Sweet No. 6883, used in the field, were inoculated with the same three cultures in the greenhouse. A1 and A2 formed galls on both of these lines in the greenhouse, and A3 produced galls on one of them (Table 6). Smut galls were produced on the five inbreds by the three cultures as a result of the inoculations made June 23. Even though there were relatively few plants in each of the lines inoculated, yet the results indicate that cultures A1, A2, and A3 differ in pathogenicity under field conditions. A type of smut infection obtained in the field as a result of the second inoculation is shown in figure 6.

Conidia injected into plants on June 2 possibly failed to come in contact with susceptible host tissue as no smut galls were formed. When the same plants were inoculated at or near their growing points on June 23 galls were produced. It is possible, also, either that environmental conditions

TABLE 15.—*Results of inoculating five inbred lines of corn hypodermically, in the field, on June 2 and 23, with three cultures of Ustilago zeae derived from the same smut gall*

	C. I. No. 133 (134)						Walden's Yellow Dent No. 452						Clark's Yellow Dent No. 440						Lancaster Sure Crop No. 311						Golden Bantam Sweet No. 6883					
	6–2–28			6–23–28			6–2–28			6–23–28			6–2–28			6–23–28			6–2–28			6–23–28			6–2–28			6–23–28		
	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls
.....	34	0	0	31	7	23	10	0	0	8	2	25	3	0	0	2	0	0	33	0	0	24	16	66	32	0	0	29	1	3
.....	33	0	0	32	16	50	14	0	0	15	8	53	7	0	0	4	0	0	27	0	0	30	10	33	35	0	0	29	8	28
.....	11	0	0	8	6	75	16	0	0	15	11	73	1	0	0	1	0	0	34	0	0	24	1	4	26	0	0	26	2	8
k ...	21	0	0	17	2	12	16	0	0	9	3	30	7	0	0	6	0	0	30	0	0	26	0	0	31	0	0	27	0	0

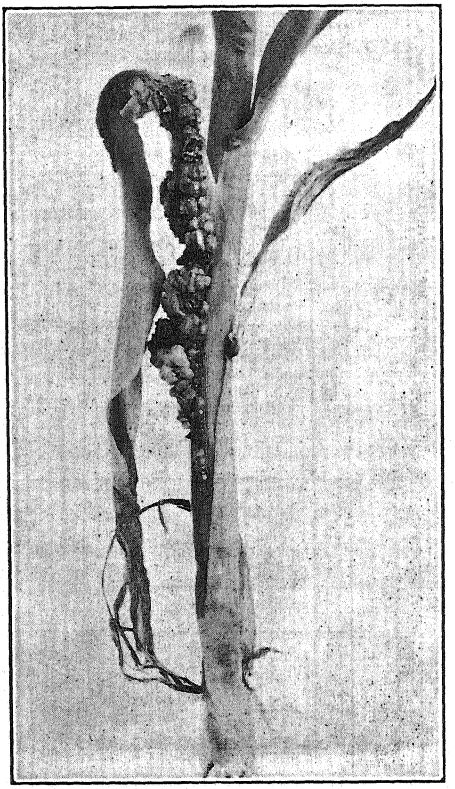


FIG. 6. Plant showing leaf and sheath infection as a result of hypodermic inoculation in the field.

were unfavorable for infection to occur from inoculations of June 2 or that the conidia did not possess the proper sexual factors necessary for gall production.

Smut galls that appeared on plants of the four inbreds, inoculated with conidia suspensions of cultures A1, A2, and A3, as is shown in table 16, were due to natural infection with the possible exception of C. I. No. 133 (134) inoculated with conidia from the A3 culture. Unfavorable environmental conditions in the field and the fact that conidia were not placed in direct contact with susceptible tissue are probably the explanation for the failure of the cultures to produce galls.

Pathogenicity of multisporidial and monosporidial cultures of U. zeae and their various combinations. Iodent No. 224a was inoculated hypodermically at two different dates with multisporidial and monosporidial cultures and their various combinations. Inoculations made on the first date showed that sporidial cultures used singly failed to produce galls and

TABLE 16.—*Results of inoculating five inbreds, in the field, on June 9 and July 2, with three smut cultures by dropping the inoculum on the apical buds of the plants*

	C. I. No. 133 (134)						Walden's Yellow Dent No. 452						Iodent No. 195						Black's Yellow Dent No. 344						Golden Bantam Sweet No. 6883					
	6–9–28			7–2–28			6–9–28			7–2–28			6–9–28			7–2–28			6–9–28			7–2–28			6–9–28			7–2–28		
	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls
.....	12	0	0	6	1	16	19	0	0	13	3	23	23	0	0	21	3	14	29	0	0	20	0	0	29	0	0	22	5	23
.....	5	0	0	4	1	25	15	0	0	11	2	18	32	0	0	33	6	18	25	0	0	17	0	0	34	0	0	30	5	17
.....	17	0	0	14	8	57	21	2	9	18	4	22	28	0	0	28	2	7	31	1	3	29	4	14	29	0	0	26	4	15
k ...	17	0	0	12	4	33	26	1	4	20	3	15	30	2	7	29	5	17	27	0	0	20	3	15	28	0	0	25	4	16

that only four combinations proved to be gall formers. These data are shown in table 17. The results of the second inoculations show that multi-

TABLE 17.—*Results of inoculating plants hypodermically, in the field, with conidial suspensions of multisporidial and monosporidial cultures and their various combinations*

Smut culture[a]	Iodent No. 224a					
	6-2-28			6-23-28		
	Number plants inoculated	Number plants with galls	Percentage with galls	Number plants inoculated	Number plants with galls	Percentage with galls
Iowa 1	10	0	0	9	8	88
Iowa 5	19	0	0	11	5	45
Iowa 7	14	0	0	14	0	0
Wisconsin 1	14	0	0	14	2	14
Kansas 38	13	0	0	14	4	29
Iowa 1 + Iowa 5	19	4	21	15	13	87
Iowa 1 + Iowa 7	16	0	0	15	14	93
Iowa 5 + Iowa 7	19	1	5	16	15	94
Wisconsin 1 + Iowa 1	17	0	0	17	14	82
Wisconsin 1 + Iowa 5	16	0	0	16	3	19
Wisconsin 1 + Iowa 7	10	0	0	10	6	60
Kansas 38 + Iowa 1	18	4	22	15	14	93
Kansas 38 + Iowa 5	18	0	0	15	14	93
Kansas 38 + Iowa 7	13	0	0	14	1	7
Wisconsin 1 + Kansas 38	17	7	41	16	7	44
Check	19	0	0	17	3	18

[a] Iowa 1, 5, and 7, multisporidial cultures; Wisconsin 1 and Kansas 38, monosporidial cultures.

sporidial cultures, Iowa 1 and Iowa 5, are evidently gall formers, as many more plants had galls when inoculated with these two cultures than did the check plants on which galls appeared as the result of natural infection. Iowa 7 did not form galls and the results do not signify that Wisconsin 1 and Kansas 38 are gall producers on Iodent No. 224a.

The results obtained under field conditions are similar to those obtained in the greenhouse, as the combination cultures were more virulent than the single cultures comprising them. This is illustrated by Iowa 1, 5, and 7 used singly and in different combinations with each other.

CULTURAL STUDIES

A comparison of the cultural characteristics of different cultures of *U. zeae* used in the preceding pathogenicity studies was made by growing

18.—*Cultural characters of twelve cultures of Ustilago zeae, from different localities in Iowa, when grown under the same conditions on carrot agar in Erlenmeyer flasks*

Age in days	Dimensions of colonies mm.	Color	Topography	Surface	Amount of aerial mycelium	Zonation	Margin
69	36 x 59	Brick red center with light purple ring grading to white at margin	Raised center, rope like; sectors present	Waxy center; cottony border	Medium	Yes	Filamentous
69	24 x 33	Purple to light brown center with white sectors	Raised center, slightly wrinkled to smooth, radially rayed; sectors present	Flaky to waxy and powdery	None	do	Not filamentous
69	23 x 27	Light brown with purple center	Raised wrinkled center, pitted slightly; sectors present	Dull waxy to ashen	Slight	do	Filamentous
69	30 x 37	Light brown to purple center grading to white at border	Raised center, pitted and wrinkled with few radial rays; sectors present	Dull waxy to cottony	do	do	do
69	28 x 44	Brownish purple center with white border	Raised center, rayed and wrinkled with deep furrows, smooth at border; sectors present	Waxy to cottony	Medium	do	do
69	42 x 42	Light purple center with white center ring	Raised center; sectors present	Cottony to velvety	Abundant	Moderate at margin only	do
69	37 x 39	White center with purple ring	Raised center, furrowed and slightly pitted, radially rayed	Waxy to velvety	do	None	do
69	40 x 45	White to purple throughout	Center raised and wrinkled, radially rayed	Waxy to cottony	Medium	do	do
69	31 x 32	Tan center with chalk white and purple sectors	Raised center, wrinkled; sectors present	Waxy to velvety	Slight	Yes	do
69	30 x 43	Purple center with tan ring; white and purple in sectors	Wrinkled and pitted center with smooth sectors	Waxy to velvety and powdery	do	do	Slightly filamentous
69	29 x 37	Deep purple center grading to brown with white border	Raised center and ridged at border; sectors present	Waxy to cottony at border	do	do	Filamentous
69	39 x 45	Tan to purple throughout	Raised center and much wrinkled to border; sectors present	Waxy to slightly powdery	Medium	do	do

them on 20 cc. of carrot agar in 200 cc. Erlenmeyer flasks. The carrot agar used in culturing each group of cultures was prepared and sterilized at the same time. After inoculation the flasks were kept at room temperature in the same locker in the laboratory so that they would be exposed to the same environmental conditions.

The data on the cultural characters of Iowa cultures 1 to 12, inclusive, at the start of the pathogenicity test previously reported in this paper, are summarized in table 18. These data are descriptive of the cultures grown on carrot agar at the age of 69 days. The colonies of the cultures differed in color, rate of growth, etc. Sectors when present varied in size and shape. No two cultures possessed identical characters. These results are comparable to those of Christensen and Stakman (3) who found that physiologic forms of *U. zeae* could be distinguished in culture. Further studies showed that colonies of the same culture, grown on carrot agar in separate Erlenmeyer flasks, for the same length of time and under the same environmental conditions, in some cases closely resembled each other and in other cases appeared to be different. Cultures 6, 8, and 12 were the most pathogenic of the twelve, and cultures 1, 5, and 7 were the least pathogenic. Their cultural characteristics do not show that any particular character or group of characters could be used as a criterion for the identification of cultures differing in pathogenicity.

Cultures A1 to A5, inclusive, derived from the same smut gall, differed from each other in their cultural characteristics, and some of these differences are noticeable in figure 7. The six monosporidial cultures differed as



FIG. 7. Cultures A1 to A5 from the same smut gall growing on carrot agar at the end of twenty-five days.

greatly from each other in their cultural characters as did any of the multisporidial cultures used in these studies.

DISCUSSION

The conclusions regarding the relative pathogenicity of the cultures of *U. zeae* used in the present investigations were based mainly on the ability of the cultures to produce smut galls on the different inbred lines which served as differential hosts. Exact differences that exist in the pathogenicity of different cultures of the fungus can be ascertained only through a statistical analysis of the data collected. To determine such differences will require the use of larger numbers of plants of each inbred line for inoculation than were used in testing the pathogenicity of the various cultures of *U. zeae* used in the present studies.

Hanna (18) reported that Mendelian factors in *U. zeae* control its sexual behavior and the expression of its pathogenicity. Researches (25, 30, 36) on the nuclear behavior of *U. zeae* indicate that a nuclear mechanism exists for the segregation and interaction of genetic factors. Seyfert (36) stated that clamp connections occurred in the vegetative mycelium of *U. zeae* and that the binucleate condition arose sometime before spore formation. Rawitscher (30) has reported that sporidia are uninucleate and give rise to uninucleate mycelia in the host plant. The single nucleated hyphal cells fuse and give rise to binucleate cells which finally become mature chlamydospores with one nucleus. Maire (25) found that the nucleus of a germinating chlamydospore always divided once and generally twice in the promycelium before sporidia were budded off. As thus outlined, the life cycle of the organism involves a nuclear fusion to form the chlamydospore and a nuclear reduction to form the sporidia. Such a nuclear mechanism forms a basis for the assumption that sexual factors controlling the degree of pathogenicity exist in *U. zeae* and are segregated out at reduction division and combined in different ways at nuclear fusion. Cultural differences that were noted between cultures grown on the same media probably were due partly to mutation (3, 39) and partly to the expression of different genetic factors carried by the cultures. Furthermore, it is possible that the physiologic reactions of the fungus are very delicately balanced and that only a slight difference in water content, acidity, etc., of the culture media was responsible for some of the differences in cultural characteristics.

SUMMARY

All multisporidial cultures of *Ustilago zeae*, including the twelve cultures derived from smut galls from different localities in Iowa, and the five cultures isolated from one smut gall, differed from each other in their pathogenicity on inbred lines of corn.

Ustilago zeae is regularly heterothallic and one monosporidial culture behaved as if homothallism also occurs in the organism. When five mono-

sporidial cultures were mated, they were found to represent four sex strains of *U. zeae*.

Paired multisporidial cultures were generally more virulent than the individual cultures comprising them.

Conidia of *U. zeae* retained their ability to produce smut when exposed to winter temperature, and cultures of the organism lost their ability to produce smut galls when repeatedly transferred.

Hypodermic injection of the inoculum into young tissues of corn plants resulted in a higher percentage of smut infection than did dropping the inoculum on the apical buds. When the latter method was used in the greenhouse, more smut was produced on plants with open apical buds than on those with closed buds. Both methods of inoculation gave a higher percentage of smut infection in the greenhouse than in the field.

Colonies of *U. zeae* from the same and different cultures differed from each other in their cultural characteristics.

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HOST RANGE AND LIFE-HISTORY STUDIES OF SOME LEGUMINOUS ASCOCHYTAE[1]

RODERICK SPRAGUE

INTRODUCTION

Leguminous plants are subject to attack by many named species of Ascochyta. Recent work (9), (10), (12), (13) makes clear the need of further study of these fungi as a group. On peas (*Pisum sativum* L.), Ascochytae, originally comprised in one species (*Mycosphaerella pinodes* (Berk. and Blox.) Stone), were made into two species with *Ascochyta pisi* Lib. distinct from *M. pinodes* which was shown to have a sphaeropsidaceous stage of its own. In addition, a third type on pea, lately made a species *A. pinodella* L. K. Jones (10), was described (13). The difference in the pathogenicity and life histories of these three pea-invading organisms is of considerable importance to the grower and to the pathologist and, since literature is replete with the citations of *A. pisi* on a wide range of hosts, it is desirous to know these fungi's true nature.

The primary purpose of this paper therefore is (1) to present to the reader an account of the life histories and pathological capabilities of present-known species of Ascochyta on legumes, (2) to list their known hosts and to make revisions in species in accordance with all information brought forth. This paper will deal principally with Ascochyta species on the following naturally infected hosts:

1. *Vicia villosa* Roth.
2. *Vicia sativa* L.
3. *V. angustifolia* (L.) Reich.
4. *Lathyrus odoratus* L.
5. *Lathyrus tingitanus* L.
6. *Vicia faba* L.
7. *Medicago sativa* L.
8. *Medicago lupulina* L.
9. *Phaseolus spp.*
10. *Pisum sativum* L.

METHODS AND PROCEDURE

In keeping with the needs of the pathologist who is dealing with living organisms the writer has here attempted to cover not only morphological but physiological characteristics of the organism under consideration. He has employed standard, easily duplicatable methods that would tend to throw light on the host ranges and life histories of the types involved. The investigation was conducted along the following lines of procedure:

1. With the aid of the microscope all available herbarium material of Ascochyta in the United States and, in addition, some foreign material were studied.

[1] The writer wishes to thank Drs. O. T. Wilson, J. J. Davis, F. R. Jones, and M. B. Linford for material help and advice throughout the course of the work. He is indebted to many for herbarium material.

2. Study of personal collections and of forms in the field.

3. Camera lucida drawings and spore measurements (50 spores from a collection) were made of all material seen.

4. Isolation of Ascochyta from various hosts, from pod, leaf, stem, and hypocotyl and maintenance of cultures in sporulating condition.

5. Study of cultures of Ascochyta on various media, the most valuable of which were potato-1 per cent dextrose agar (13), and pea-soup agar.[2]

6. Inoculation studies of extensive nature on aerial and subterranean parts.

7. Attempts to develop ascigerous stages.

One of the first problems in a work of this kind is to determine just what factors, characters, and activities of the various fungi shall be employed in separating them into taxonomic divisions. The writer has used what he considers reliable mycological characters on host material as a basis, making use of both natural and, with due caution, artificial infections. He has also conducted cultural studies on artificial media in the determination of species. It is his opinion that, since fungi are so variable, it is not desirable to give technical names to varieties and forms, especially in this work where so many intermediate stages occur.

HISTORICAL

This paper, which deals primarily with available North American species, will omit mention of many familiar foreign hosts and Ascochyta species.

Types on Vicia species. Libert (11) describes *Ascochyta viciae* on *Vicia sepium* as the cause of a subrotund spot with a darker margin than the paler interior and showing epiphyllous, aggregated pycnidia (90–100 μ in diameter) containing hyaline 1-septate spores 12–15 x 4.5 μ. The description is very similar to that for *A. pisi* (13).

Cooke (3, p. 115) attempted to place Libert's specimen under his *Phyllosticta viciae* but seems to have been concerned with an immature Ascochyta.

Trail (29) describes *A. vicicola* (Trail) Sacc. on *V. sepium* from Scotland and, except for its slenderer spores (13–16 x 2.5–3 μ), the description sounds similar to *A. viciae* Lib.

Sydow (27), (28) describes *A. vicia-lathyroidis* from Germany. He also names *A. ervicola* on *Vicia hirsuta* S. F. Gray.

Spegazzini (23, p. 321) describes a dark yellowish to brown leaf spot on *Vicia faba* from Argentine. His species *A. fabae* is characterized by large spores (10–25 x 5–6 μ).

[2] Pea soup agar was made by adding the contents of an 8 ounce can of Campbell's pea soup to 2 liters of distilled water, solidified with 1.3% agar, filtered, tubed, plugged, and sterilized at 15 lbs. for twenty minutes.

Among extensive and more recent work we find Stone (24) including both *A. viciae* Lib. and *A. vicicola* under *Mycosphaerella pinodes* and Scalia (22), even more sweeping, in grouping practically all the forms on peas, vetches, and beans under *A. pisi* Lib.

Recently, it was pointed out by Linford and Sprague (13) that an Ascochyta spot was of common occurrence on *Vicia angustifolia* in Wisconsin and that hosts and culture symptoms (potato-2 per cent dextrose agar) bore close resemblance to *A. pisi* and none to cultures of *M. pinodes*.

Both Stone (24) and Atkinson (1) found reason to conclude *M. pinodes* occurred on vetch in that an ascigerous stage identical to *M. pinodes* was found on hairy vetch.

Types on Lathyrus species. Trail (29) described *Ascochyta lathyri* on *Lathyrus silvestris* as causing an ill-defined, often extensive leaf spot bearing numerous small pycnidia that contained spores measuring 8–10 x 2.5 μ. Saccardo (19) notes them still smaller in his work, 5–8 x 2.3 μ.

Stone (25) in describing *Mycosphaerella ontarionenis* stresses the small size of the pycnidiospores.

A large-spore Ascochyta, *A. orobi*, has been described on *Lathyrus vernus* by Saccardo (18, p. 303). Harkness (7) gives *A. pisi* on the leaves of *L. palustris* from California.

Bubák and Kabát (2) described *A. lathyri* var. *lathyri-odorati* as the cause of light colored to brownish spots of a general, indefinite nature on the leaves of the sweet pea. Spores were small, 8–14 x 2.5–3.5 μ.

Types on Medicago species. Ascochyta imperfecta Pk. (14), (26), (4) is considered the chief Ascochyta species on alfalfa in this country. It differs from *A. medicaginis* Bres. in smaller, frequently non-septate, spores.

Rostrup (17) from Denmark has mentioned *A. pisi* on alfalfa and Saccardo (20) describes *A. pisi* var. *medicaginis* on living stems of alfalfa from Spokane, Washington.

SYMPTOMATOLOGY

Types on Vicia species. Without exception leaf-, stem-, and pod-spot material on *Vicia villosa, V. sativa,* and *V. angustifolia* showed light buff to white spots (4–10 mm. diameter), definitely red or brown-bordered and having prominent pycnidia usually concentrically zoned.

On *Vicia faba* the type seen (leaf spot from California) appeared as large spots, light-centered with a tendency towards shot-holing and bordered by a discolored, black, sometimes red border. The large pycnidia were prominently arranged in concentric order. Infected seed of this host showed sunken brown to black spots up to a centimeter in diameter.

Types on Lathyrus species. Most of the work was done with material on sweet peas (*L. odoratus*). Material from Minnesota showed vines cov-

ered with purple to brown stem lesions in which many black-brown small pycnidia occurred. Leaf spots were vague, indefinite light brown to pale tan in color.

Types on Medicago species. On alfalfa (*Medicago sativa*) injury is (except for material collected by Simmons in Wyoming and Washington) confined to the sides and tips of leaves and petioles as indefinite diffused brown bordered spots with lighter centers and obscure pycnidia.

On *M. lupulina* spots are very light with narrow but definite borders and large pycnidia, fairly prominent.

ETIOLOGY

Types on Vicia species. Because of the confusion relative to this group it seems well to list a considerable number of specimens with brief notes where it is deemed advisable. Measurements are of mean spore size when not otherwise given.

Vicia villosa Roth.

J. M. Bates (U. S. D. A.), Nebr., 1897. Spores broader proportionately than most of the collections to follow, 14.6 x 4.3 μ; Barth.: Fungi Columb. 1506, Alabama, 1901, pod spot, 14.6 x 4.1 μ from specimen at Ohio State Univ., 13.7 μ x 3.2 μ from specimen at Wash. State College; Barth.: Fungi Columb. 2305. (The host *Vicia americana* Muhl. seems to be *Vicia villosa* Roth. from material in the Bureau of Plant Industry Herbarium at Washington, D. C., 13.7 x 4.3 μ; Clinton, Mass., 1906, spores cylindrical with homogenous contents, 12.7 x 3.9 μ; Clinton, Granby, Conn., 1907, 13.8 x 4 μ; J. J. Davis, Wis., 1914, 13.6 x 3.7 μ, spots seem immature with greenish, water-soaked appearance noted in stages of inoculated infection development; R. M. McKee (U. S. D. A.), Fayetteville, Ark., 1919, pycnidia 70–100, spores 13.2 x 3.6 μ; R. M. McKee, Georgia, 1921, 14.8 x 3.9 μ; J. H. Miller, Georgia, 1923, leaf spot, spores cylindrical, contents clear, hyaline 13.8 x 4 μ; Linford, N. J., 1928, R. F. Poole, N. J., 1924, pod spot, spores cylindrical, blunt ends, 13.6 x 3.6 μ; Kienholz, Pullman, Wash., 1927, leaf spot, spores, 12.5 x 4.3 μ, pod spot spores, 13.1 x 4.2 μ; Sprague, Ohio, 1928, 13.4 x 4.3 μ; Sprague, Ohio, 1928, 13.4 x 4.5 μ; Wolf, N. Y., spores slender, 12.9 x 3.4 μ; Vestergrun, Microm. rar. sel. 1537. Suecia, 1898, slender, cylindrical spores with homogeneous contents, 15.3 x3.7 μ.

Vicia sativa L.

Dearness, Ontario, 1897, spores clear, cylindrical, 15 x 4.5 μ, pycnidia 60–100 μ diameter; Tranzschel et S. Myc. Rossica 37, pod spot, pycnidia arranged concentrically, spores large, frequently several septate, similar to material from Mississippi, 16.6 x 4.7 μ; J. H. Miller, Ga.; L. E. Miles, Miss., 1922, 14.1 x 4.3 μ; H. H. Wedgeworth, Miss., 1927, spores large,

variable, cylindrical, somewhat bulgy, especially the frequent two or more septate ones, 16.1 x 4.6 μ (13–19 x 3.5–6 μ), pycnidial walls loosely structured, light golden, pycnidia of comparatively large size.

Vicia angustifolia (L.) Reich. and varieties.

H. M. Barre, Clemson College, S. C., 1909, 13.4 x 4.1 μ; Kabát et Bubák Fungi Imp. Ex. Ger. (Labelled *A. vicia-lathyroidis* Syd., 13.7 x 4.3 μ; R. M. McKee (U. S. D. A.), Ga., 1921, 15.4 x 4.1 μ, many are more than one-septate and range up to 22 μ in length; J. J. Davis, Barneveld, Wis., 1921, spores cylindrical and not constricted in some, strongly so in others, 14.2 x 4.2 μ; Davis, Ridgeway, Wis., 1921, spores cylindrical, 13.4 x 4.2 μ; L. E. Miles, Miss., 1922, 13 x 4.5 μ; J. H. Miller, Ga., 1923, 15.6 x 4.1 μ; Linford, Wis., 1924, spores cylindrical, some two-septate, 15 x 4 μ, in culture on potato-2 per cent dextrose agar this resembled *A. pisi* from the pea.

Vicia faba L.

F. R. Jones, Corvallis, Oregon, 20.3 x 4.3 μ; R. M. McKee, Chico, Calif., 17.3 x 4.7 μ.

While great variation in size may be found both in spores and pycnidia, even in the same collections, those of the Ascochyta on vetches range distinctly larger in size over those of *A. pisi* on the pea. The smallest are probably those on *V. villosa* and all transitions occur between these and the large type on *V. faba*. Under the oil-immersion lens the pycnidia of the latter are light golden in color and show a complex of intertwining mycelium which is not quite so compact as that of the firmly knit pycnidial stage of *M. pinodes* on pea. There is a tendency away from the formation of an outer wall marked off in squares or polygons and the ostiole is less definite.

Types on Lathyrus species. Spores of material collected by Vande Vere from Hopkins, Minnesota, on *L. odoratus* were small, plump, cylindrical to faintly fusiform and had a mean spore size of 9.4 x 3.6 μ (Pl. XXXV, I).

Infected pod material of *L. odoratus*, sent by F. R. Jones from Madison, Wis., showed spores and pycnidia identical to *M. pinodes* on one pod but on the others of the collection the material was indistinguishable from the Minnesota collection.

Types on Medicago species. Material examined from eleven widely scattered States show the spores of *A. imperfecta* Pk. on alfalfa to average 10.6 x 2.8 μ to 13.4 x 4.3 μ, mean spore size, with a grand average for the fifteen collections of 12.1 x 3.4 μ. The spores vary considerably in appearance. Mature material from Ohio is made up mainly of septate, cylindrical spores with homogeneous contents. Material from the west gave a larger percentage of immature and non-septate pycnospores (Pl. XXXVI, A, B, C, E). Co-type material collected by French closely resembled this western material.

PURE CULTURE STUDIES

All of the work was done with potato-1 per cent dextrose agar unless otherwise stated. Growth at room temperature.

Types on Vicia species. 1. *Vicia angustifolia* produces a white to buff fluffy growth very similar to *Ascochyta pisi* from pea (10), (13) with the production of carrot-red spore masses and the formation of pigment in the substratum (13).

2. *V. villosa.* Similar to above but some tendency to darker coloration in some isolations (New Jersey and Washington) approaching an olive color in some saltations. Rate of growth on various agar very close to *A. pisi* from pea (13). On agar long cylindric spores are produced, quite in contrast to the short cylindric ones on the host. (Compare Pl. XXXIV, E, F, H, J, K, M). Pigment occurs in substratum.

3. *V. faba.* Very similar to *A. pisi* on pea, less spore production, spores 14–21 x 4–6 μ, carrot-red in mass. Pigment in substratum.

Types on Lathyrus species. 1. *Lathyrus odoratus.* Material from Wisconsin, seed and pod. Rapid grower, slightly faster than *A. pinodella* (13). Forms a flat grey growth with frequent so-called saltations of lighter or sometimes darker strips and accompanied by the production of abundant small black pycnidia buried in the substratum, with only the upper half protruding. The substratum is made up of chlamydospores and dark compacted hyphae. The few tendrils of spores exuded are pale flesh to grey in mass and are made up chiefly of non-septate members. (To compare *A. pinodella* and *A. lathyri* as to spore characters (Pls. XXXV, I and XXXVI, F, G).

2. *L. tingitanus.* From foot-rot material sent by Dr. Weimer from Mississippi. Identical with *Mycosphaerella pinodes* (13).

Types on Medicago sativa. Isolations from various parts of the country, while varying greatly in morphology on the host, were indistinguishable in the test tube. Growth flat grey, felty with olive-brown patches. The felty growth is gradually superseded by a darker, flatter, carbonaceous growth in which numerous small black pycnidia are imbedded. The substratum consists of masses of chlamydospores and stromatic hyphae. The remains of the original felty growth, plus additional later tissue, obscures the pycnidia with a thin evanescent mycelial felt. Viewed from beneath, a plate culture presents an olive-brown appearance. Pycnidia exude pale pink spore masses made up largely of nonseptate spores. Rate of growth is rapid, paralleling that of *A. pinodella.*

On Melilotus stems, in culture, *A. imperfecta* produces only red-brown carbonaceous pycnidial masses that produce spores in quantities. Spore production may be obtained wholesale by use of water suspension inoculations on the surface of dextrose-potato agar or pea-soup agar.

INOCULATION STUDIES

In the work as many of the economic legumes grown in this country as time would permit were employed, including practically all those for food and forage, as well as a number of ornamental types.

The following types of Ascochyta were employed in inoculation work.

1. *A. pisi* from pea (6 isolations).
2. *M. pinodes* from pea (5 "). (One from *L. tingitanus.*)
3. *A. pinodella* (4 ").
4. *A. sp.* (*lathyri*) from *L. odoratus* (1, from Wis.)
5. *A. sp.* (*pisi*) " *V. villosa* (2 isolations).
6. *A. sp.* (*pisi*) " *V. faba* seed sent to the writer nine years after harvest in California (F. R. Jones). Fungus and seed both viable.
7. *A. imperfecta* from alfalfa (2 isolations).

Methods of inoculation of aerial parts. Plants were grown in four-inch pots in the greenhouse, from selected seed; and, when three weeks old, they were moistened under the tap and thoroughly sprayed with vigorous pycnospores in water suspension.

After inoculation the technique employed was similar to that described in previous work (13):

1. Incubation at 18–23° C. in spray chamber for 48–60 hours.
2. Use of check plants in all cases.
3. Plants removed from chamber and left in damp place for symptoms to develop; these were recorded daily.

TABLE 1.—*Results of inoculations on Vicia species (aerial).* (*Given in number of pots showing positive or negative.*)

	+1[3]−		+2−		+3−		+4−		+5−		+6−		+7−	
V. angustifolia	1	3	1	1	3				2	1		1		1
V. atropurpurea	1	2	2	1	3	1		1	2		1	2		
V. cracca		2	1		3	1		1	1	1	1			1
V. dasycarpa		3	1	1	3	3			1		1			1
V. ervilia	3	1	1	1	2			1	2		2	1		2
V. faba (2 types)	3	9	2	4	2	3		1	2		2	1		
V. monanthis	1	1	3	1	1		1	1	1		1			1
V. pannonica		6	2		2	2		1		2		1		3
V. villosa	5	8	2	3	3	4	1	1	5	1	1	5		2
V. sativa (2 types)	8	9	2	1	5	1	1		3	1	4	1		2
Total	22	44	17	13	27	15	3	7	19	6	13	12	0	13

[3] Number corresponds to number given source of culture at beginning of inoculation studies, for example, 1, *A. pisi* from pea, 2, *M. pinodes* from pea, etc.

4. Reisolations made and results recorded.

Methods of inoculation of subterranean parts. The various cultures were grown on sterilized peas in tubes and this material powdered and mixed with sterile soil in the same manner employed in previous work (13).

Results of Aerial inoculations. Out of some nine hundred odd pots of inoculated legumes, comprising sixty species and varieties of hosts, there were many that proved negative to attack from all Ascochyta species. Important among these were the following: five varieties of garden beans (*Phaseolus vulgaris*), soy bean, hyacinth bean, rice bean, and white and crimson clover.

On Vicia species. The results are given in table 1.

From the results obtained it indicated that most of the vetches are resistent to *A. pisi* from pea and are considerably more susceptible to Ascochyta isolated from vetches. A comparison with a summary of results on six varieties of peas shows this definite physiological trend. The figures 1 to 7 again refer to source of inoculum.

	1		2		3		4		5		6		7	
	+	−	+	−	+	−	+	−	+	−	+	−	+	−
Number of pots.....	26	0	23	0	20	0	3	4	2	14	2	14	3	12

Inoculation studies brought out the fact that the blackish border on spots on *V. faba* was not due to fungal characters differing from those on other vetches but was due to a tendency of this particular host to blacken on injury. It may be compared to the habit of *Baptisia bracteata* (Muhl.) Ell. blackening on curing. Subtantiation of these statements was shown by the fact that Ascochyta isolated from *V. faba* developed light-centered red-bordered spots on other vetches.

In the vetch inoculations the spores and pycnidia retained their general characteristics through the several cycles of isolations and inoculations attempted. For example, the mean spore size of the large-spore Ascochyta from *V. faba* continued to be over 18 x 5 μ and the others somewhat smaller.

On Lathyrus species. The results are given in table 2.

Symptoms of inoculations from Ascochyta on *Lathyrus odoratus* are variable. Sometimes it causes dark brown lesions and leaf spots similar to those of *A. pinodella* and again, on the sweet pea, infection results in indefinite leaf and stem-tip injury similar to that given in literature for *A. lathyri* on various hosts. This condition is possibly due to rapid spread of the fungus in the succulent tip of the plant.

On Medicago, Trifolium, and Melilotus species. Alfalfa and bur clover (*Medicago hispida*) were immune from everything except *A. imperfecta* which was 100 per cent effective on them.

Melilotus indica, M. officinalis, T. hybridum, and *T. pratense* were negative to everything except about 20 per cent of the cases where inoculated

TABLE 2.—*Result of aerial inoculation of Lathyrus species. (Results given in number of pots positive or negative.)*

	+1[4]−		+2−		+3−		+4−		+5−		+6−		+7−	
L. clymaceum	2				1		1		1			2		2
L. latifolius		2		1	1	1	3	1		1				
L. odoratus	3	7		5	4	3	8	1	1	1	2	2		4
L. sativus	1		2		3		2	1	1		1	1		1
L. tingitanus		5	4		2		2	1	1	1		3		3
Total	6	14	6	6	11	4	16	4	4	3	3	8		10

[4] Numbers 1 to 7 refer to source of inoculum, see first paragraph under Inoculation Studies.

with *A. imperfecta.* The injury was in the form of a leaf scorching and rarely could be determined as Ascochyta injury except through reisolation.

On Phaseolus species. *P. aureus* and *P. aconitifolius* were susceptible in part to all pea-borne Ascochyta, but *P. mungo* and *P. multiflorus* were negative except for a small amount of *A. pinodella* in one inoculation. *P. lunatus* showed doubtful infection here, also. *P. aconitifolius* showed one case of infection with *A. imperfecta.*

On Miscellaneous hosts. Among various susceptible hosts not previously mentioned *Vigna sinensis, Cicer arietinum,* and *Lupinus polyphyllus* showed some susceptibility to *Mycosphaerella pinodes* and *Ascochyta pinodella.*

Length of period of incubation under conditions of inoculations.

Fungus	Host	Length of time required
Ascochyta pisi	pea	5–7 days
" (*pisi*)	*Vicia villosa*	do
" (*pisi*)	*V. faba*	do
" *pinodella*	pea	$2\frac{1}{2}$–$3\frac{1}{2}$ days
" (*lathyri*)	*L. odoratus*	3 –5 days
" *imperfecta*	*Medicago sativa*	3 –5 days
M. pinodes	pea	$2\frac{1}{2}$–$3\frac{1}{2}$ days

Results of subterranean inoculations. The results of some four hundred pots of plants were very inconclusive in the main. It was found, however, that *M. pinodes* could cause severe injury to *Cicer arietinum* and slight to moderate damage to vetches and Lathyrus species. *A. pinodella* was able to injure some of these types also, especially *Lathyrus tingitanus.*

A. pisi from pea and *Ascochyta* (*pisi*) from *Vicia villosa* seemed to be largely negative although there was some indication of a slight amount of a pale cortical rot.

ATTEMPTS TO DEVELOP ASCIGEROUS STAGES

Methods. Use of Melilotus stems and oat agar. Inoculation work done with potted plants. The plants were left for varying periods under green-

house and overwintering conditions. All of the pure cultures in stock were tried.

Results—Negative on all cultures of *Ascochyta pisi* from pea, Ascochytae from all vetches, *A. pinodella* from pea, *Ascochyta* (*lathyri*) from *Lathyrus odoratus* and *A. imperfecta* from alfalfa.

Perithecia answering to those of *Mycosphaerella pinodes* were obtained from such material isolated originally from pea, *Lathyrus tingitanus,* and *L. odoratus.*

DISCUSSION, OBSERVATIONS, AND CONCLUSIONS ON ASCOCHYTA ON VICIA SPECIES

The writer, being unable to obtain material of Stone's Mycosphaerella on vetches, has never observed any Ascochyta on vetches that does not fall close to *Ascochyta pisi* Lib. The symptomatology, absence of a perfect stage, cultural characters on media, and taxonomic traits seem to place the vetch forms in this country with *A. pisi.*

The extremely large spores of *A. pisi* on *V. faba* (Pl. XXXIV, A, B, C), are, together with their pycnidia, somewhat distinct from *A. pisi* as it occurs on the pea. Saccardo and Marchal (21) gives *A. pisi* f. *foliicola* with spores 15–18 x 4–5 μ on peas. Saccardo later (18, p. 303) thought this related to *A. boltshauseri* Sacc., a form usually found on Phaseolus spp. in Europe. Study of the California *V. faba* material and the brown, distinctly zonate spot caused by *A. boltshauseri* shows nothing in common (Pl. XXXVI, P, for spore characters of *A. boltshauseri*). Considering the great resistance of beans (*Phaseolus vulgaris*) to all types of *A. pisi,* we are safe in eliminating *A. boltshauseri.*

Various large-spore Aschochytae are listed on vetches. Jaap (8) mentions *A. viciae* on *V. sepium* with spores 18 x 5 μ. *A. orobi* Sacc. occurs on vetches as well as Lathyrus, according to European collectors. From German material seen and descriptions in literature the writer considers this species in part, at least, identical to *Ascochyta pisi* on vetches in this country.

Transitional types between those on the pea and the broad bean are common on *V. sativa* (Pl. XXXV, D, E, F), and on *V. angustifolia* Pl. XXXV, G) spores 15–25 μ in length are commonly found. Since it would be impossible to make more than highly artificial dividing lines between these types the writer considers that all forms on vetches with the typical symptoms of *A. pisi* on pea should be placed with this species.

Examination of type material sent through the courtesy of Drs. Sydow and Samuelsson of *A. ervicola* and *A. vicia-lathyroidis* leads the writer to place these under synonymy with the other types on vetches (Pl. XXXV, B, C, H). Peculiar slender-spore Ascochytae on vague, light tan spots on leaves of *V. caroliniana* and *V. americana* sent to the priter by Dr. J. J.

Davis do not seem to belong with the others and are left unclassified (Pl. XXXV, A).

The fruiting bodies of *A. pisi* on *V. faba* approach the pseudopycnidia of Potebnia (16), Diedicke (6), and others. Diedicke bases his genus Stagonosporopsis on the presence of loosely formed pycnidia, often acervulus-like, and the production of a variable percentage of multiseptate spores. An examination of many cultures and of host material and a study of *A.* (*Stagonosporopsis*) *boltshauseri* (5) (15) leads the writer to believe that the term pseudopycnidium is not valid for this particular group and that the genus Stagonosporopsis is untenable.

Technical description of Ascochyta pisi Lib. It seems advisable to give a revision or extension of the description of *Ascochyta pisi* to cover the range indicated in this paper. On account of small differences noted throughout the descriptions on various hosts, it will be necessary to divide this voluminous description into three parts for the sake of clearness and usefulness.

Ascochyta pisi Lib. on *Pisum sativum* L. and varieties. Spots definite, circular on leaves and pods, elongate on stems, yellowish brown with dark brown to reddish border which is often prominent, sometimes whitish in center, spots not defined on mature plant parts; pycnidia gregarious at center or in circular zones, somewhat prominent, depressed-globose, light golden brown to darker brown, ostiolate, 75–225 μ; spores hyaline with homogeneous contents and occasional oil drop, oblong, ends rounded, straight or somewhat curved, 1 (1–3) septate, somewhat constricted, 10–14 (6–19) x 3–5 (2.5–6) μ. Mean spore length usually less than 13.5 μ (12–15).

On leaves, stems, pods, and seeds of *Pisum sativum* L. and on aerial parts of *Lathyrus spp.* and *Vicia spp.* by artificial inoculations.

Ascochyta pisi Lib. on Vicia species. Spots definite, circular, occasionally elongate on leaves and pods, elongate on stems, at first yellowish brown to green, later white with dark red to brown border which is always prominent when mature, spots not defined on mature or senile parts; pycnidia gregarious at center or in circular zones, very prominent, depressed-globose, hyaline (light golden) to dark brown (pycnidia on stem parts often dark brown), ostiolate, 60–225 μ; spores hyaline, contents when mature homogeneous with rare oil drops, 1 (1–4) septate, cylindrical, sometimes curved, ends rounded, scarcely to somewhat constricted, 10–17 (8–25) x 2.5–5 (2–6) μ, mean spore length variable.

On leaves, stems, pods and seeds of *Vicia spp.* and by inoculation on aerial parts of *Lathyrus spp.* and slightly on *Pisum sativum.*

Ascochyta pisi Lib. on *Vicia faba* L. Spots usually definite, circular to elongate on leaves and pods, elongate on stems, at first dark brown or olive to black, later dirty yellow to white in center with dark brown to red border which is often prominent though sometimes diffused; pycnidia gregarious near center or in circular zones, very prominent, depressed globose, light brown, sometimes darker, parenchyma cells of walls usually not closely

packed, ostiolate, 80–250 μ; spores hyaline, contents homogeneous except frequent medium to large oil drops and crystalline-like material, 1 (1–4) septate cylindrical straight or somewhat curved, constricted at septum (sharply constricted in spores more than one-septate), ends rounded, 14–25 (12–28) x 3.5–6 (3–7) μ, mean spore length usually greater than 17 μ when mature.

On leaves, stems, pods and seeds of *Vicia faba* L. from Oregon and California and by artificial infection of other Viciae.

DISCUSSION, OBSERVATIONS, AND CONCLUSIONS ON ASCOCHYTA PINODELLA

From extensive collections recently sent to the writer by M. B. Linford and others the prevalence of *A. pinodella* in the United States is an established fact. It seems to be the commonest pisicolous Ascochyta in the arid regions of the West and it is found often in the East. Both this species and *A. pisi* were found associated with *Peronospora viciae* (Berk.) De Bary on pea leaves in a number of Linford's collections. The occurrence of Phoma or Phoma-like forms was commonly noted in some of Linford's material. Their nonpathogenicity was determined. Their presence may confuse.

The writer has found no collection of *A. pinodella* on anything except peas. This is surprising in view of the fact that some of the vetches are extremely susceptible to the organism under greenhouse conditions of inoculation. While hairy vetch and Hungarian vetch (*V. pannonica*) show a high degree of resistance, spring vetch (*V. sativa*) is nearly always killed to the ground. Pycnidia develop sparsely in some cases and with great profusion in others.

The very obscure nature of the common symptoms, those of a foot rot (in the main), no doubt allows many cases of this Ascochyta's depredations to pass unobserved.

DISCUSSION, OBSERVATIONS AND CONCLUSIONS ON MYCOSPHAERELLA PINODES

Through inoculation work it was shown that *M. pinodes* could attack a variety of hosts under the conditions of the experiment and often with great vigor. Its absence on vetches can be attributed, the writer believes, to a late-season parasitism that has not been observed by most workers who collected earlier in the summer. No doubt this is the explanation of Stone's material. It is known that rainy weather towards harvest may result in severe blighting of peas. Probably *M. pinodes* on pods of *L. odoratus* was a late September condition. Total negativeness of inoculation work on *L. odoratus* with cultures of *M. pinodes* bore this out.

The presence of *M. pinodes* in foot-rot material of *Lathyrus tingitanus* was clearly of a pathogenic nature.

DISCUSSION AND CONCLUSIONS ON ASCOCHYTA LATHYRI

Although similar to *Ascochyta pinodella* in several respects the small-spore Ascochyta on sweet pea in this country shows distinct spore characters, different period of incubation, different host range, and different cultural characters. In spite of lack of proof of connection with *Mycosphaerella ontarionensis* the writer considers the material to go under *Ascochyta lathyri*. (Pl. XXXV, K, L, M, N; Pl. XXXVI, F, G, for comparison of types.)

DISCUSSION, OBSERVATIONS AND CONCLUSIONS ON ASCOCHYTA IMPERFECTA

There can be little doubt that *A. imperfecta* is distinct from *A. medicaginis* as shown by inoculation work, coupled with study of material. Work with cultures of the latter as well as comparison with European types on alfalfa is necessary. (Pl. XXXVI, I, J.)

The surprising range of *A. imperfecta* in inoculation studies is not so remarkable when one considers that pycnidia and definite symptoms developed only on alfalfa and bur clover. The other hosts were plainly resistant and considering the young condition of the plants and ideal infective conditions it is rather doubtful if *A. imperfecta* would be found on these various hosts in the field. Bur clover no doubt may carry the trouble.

The variability of *A. imperfecta* in the field seems to be due to environment and not stable morphological differences. Under cultural conditions on media and through inoculation work as pointed out the various isolations were practically identical. (Compare Pl. XXXVI, A to E.)

The material collected by Simmons from Wyoming and Spokane, Washington, and referred to *A. pisi* var. *medicaginis* (Pl. XXXVI, H) by Saccardo is confusing. While spore characters are not unreconcilable with *A. imperfecta* the position of the pycnidia on the stem is unusual. Fresh material is needed before the position of this type can be made certain. The fact that the pycnidia on the stem of alfalfa are black and carbonaceous is due to their position and not to any morphological distinction. Extensive work with inoculations shows that, from a single inoculation, pycnidia may be obtained on the leaf that belong to Ascochyta and on the stem of the same plant that belong to Diedicke's genus Diplodina (6).

The writer would be inclined to place the material on alfalfa collected by Simmons under *A. imperfecta* with a question mark.

OBSERVATIONS ON ASCOCHYTA PHASEOLORUM SACC.

Two collections from Takoma Park, D. C., by A. Arnes on *Phaseolus lunatus* L. (*P. limensis*) show progressively greater increase in the percentage of septate spores (20 per cent) in August collection, 70 per cent, October) of material labelled *A. pisi*. The writer has compared this Takoma

Park material with foreign collections and finds it close to *A. phaseolorum*. The spores were short, cylindric, 7 x 2.5 μ mean spore size and the pycnidia were large, 135–180 μ. The spores were clearly septate and the cross walls were not to be confused with the false septations of the Phomopsis stage of *Diaporthe phaseolorum* (Pl. XXXVI, M to P).

Incidental work not to be presented here showed clearly that *Ascochyta phaseolorum* Sacc. and *Phyllosticta phaseolina* Sacc. were distinct.

SUMMARY

1. This paper is, in the main, a taxonomic study of Ascochytae on Leguminosae but treats of the life histories and physiological characteristics of the various forms and species as well as the mere observation of their morphological features.

2. Most of the North American collections on Viciae were shown to be similar or identical to *Ascochyta pisi* as it occurs on the pea. A form of *A. pisi* on the broad bean (*Vicia faba*) resembles *A. fabae*. Its difference in symptomatology from *A. pisi* on other vetches was shown to be due to host reaction. No ascigerous stage was found with any form of *A. pisi*.

3. Inoculation studies show *Mycosphaerella pinodes* to be pathogenic on a wide range of hosts, a fact not borne out by field and herbarium study. The writer has found this organism only on *Pisum spp., Lathyrus odoratus* and *L. tingitanus*. Stone's material on vetches was not examined. It is suggested that the occurrence of *M. pinodes* on vetches may be limited to late season.

4. A study of *A. pinodella* shows it to be common and widespread on the pea in America and under greenhouse conditions able to attack many hosts not found under field conditions as yet.

5. *Ascochyta lathyri* is the common species on *L. odoratus* in America.

6. *Ascochyta imperfecta* on alfalfa was shown to be distinct from *A. medicaginis* on *Medicago lupulina* and, while variable in the field, is clearly one species as far as leaf-spot symptoms go.

7. *A. phaseolorum* has been collected in America.

8. The genus Stagonosporopsis is invalid and Diplodina is of doubtful value.

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CORVALLIS, OREGON.

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EXPLANATION OF PLATES

PLATE XXXIV

A. *Ascochyta pisi* on *Vicia faba*, leaf spot. Corvallis, Oregon.
B. *A. pisi* from *V. faba*, leaf spot caused by artificial inoculation with culture of pycnospores from California *V. faba* seed.

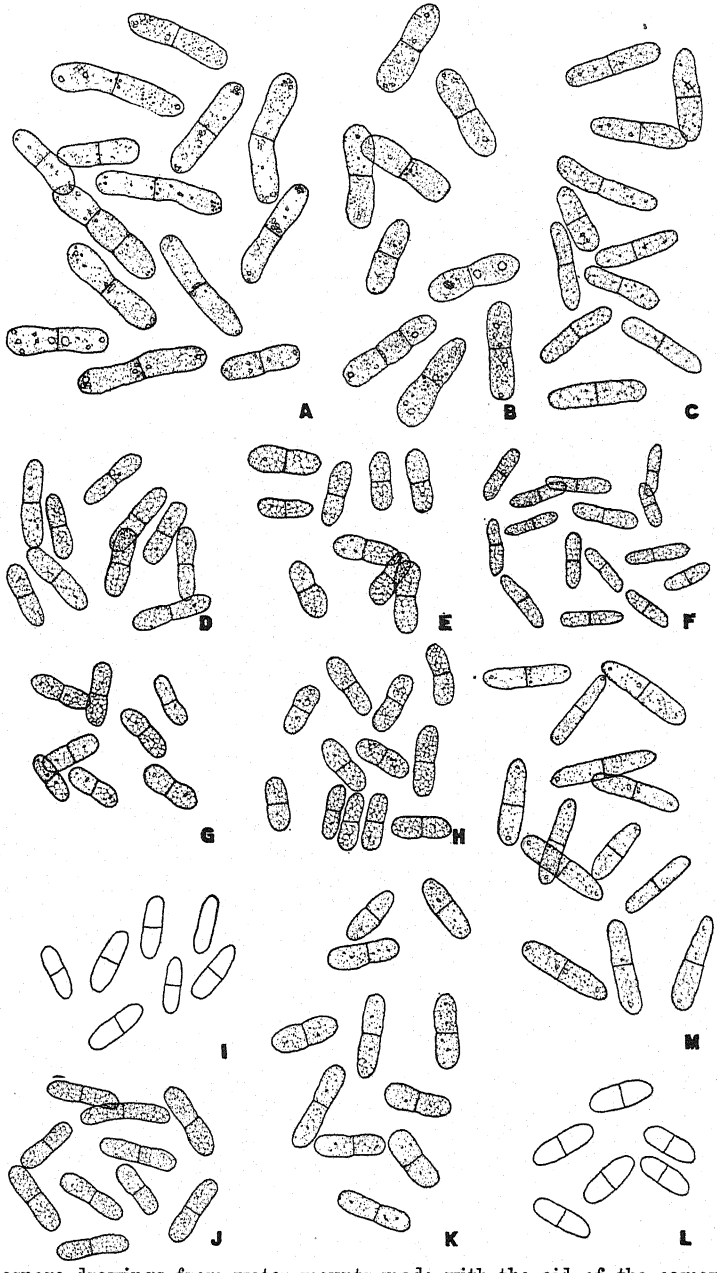
C. *A. pisi* from *V. faba* grown on potato-dextrose agar.
D. From artificially inoculated spot on *V. faba* with dextrose agar culture of *A. pisi* isolated from pen.
E. Same as D but inoculum obtained from *V. villosa,* Pullman, Wash.
F. From culture of *A. pisi,* isolated from pea, on potato-dextrose agar.
G. From artificially inoculated spot on *V. sativa* with *A. pisi* from pea.
H. *A. pisi* from leaf spot on *V. villosa* (Pullman, Wash.).
I. Same type as fig. H, from suecia, collected by Vestergrun.
J. On potato-dextrose agar, material from fig. H.
K. Same as J but source is Cincinnati, Ohio.
L. *A. pisi* on *V. villosa,* leaf spot. Valentine, Nebr., J. M. Bates. 1897.
M. On potato-dextrose agar, material from fig. H.

PLATE XXXV

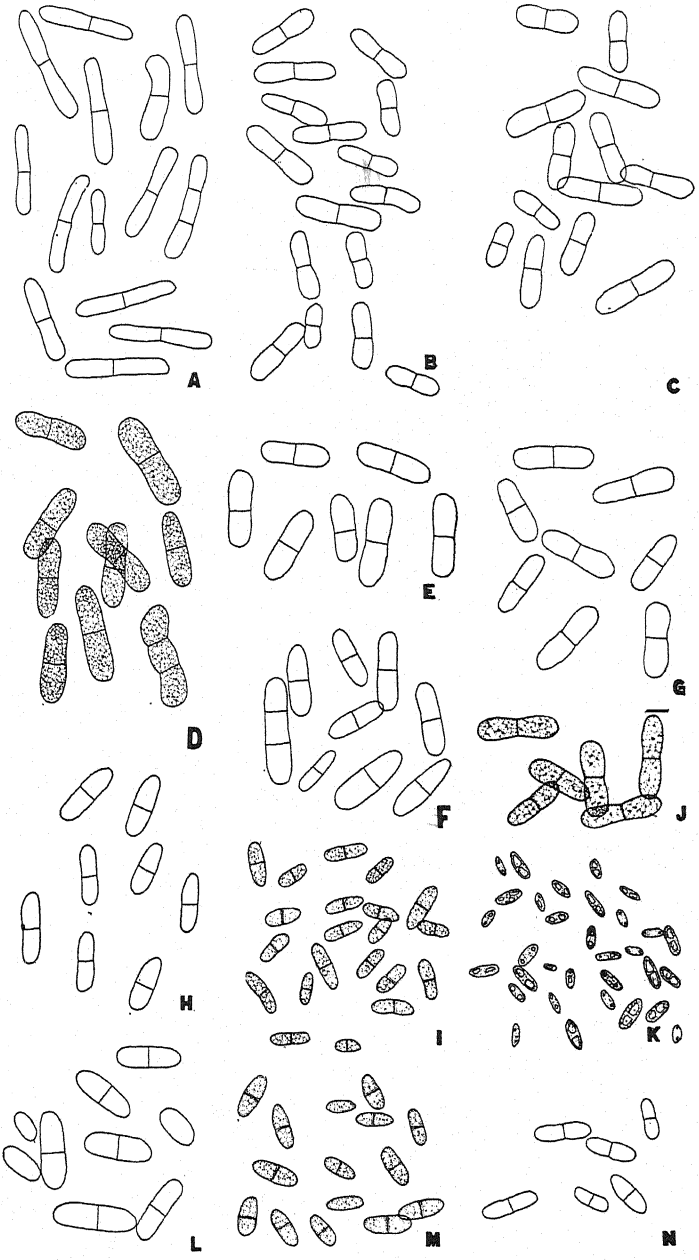
A. Ascochyta species on *V. Americana* from Wisconsin (J. J. Davis).
B. Type material of *Ascochyta ervicola.*
C. Type material of *A. vicia-lathyroidis.*
D. *A. pisi* on *V. sativa.* Leaf spot, Mississippi.
E. Same as D from London, Ontario (Dearness).
F. Same as E from Russia (Myc. Rossica 37).
G. *A. pisi* on *V. angustifolia* from Wisconsin.
H. *A. pisi* on pod of *V. hirsuta* from Maine.
I. *A. lathyri* on *L. odoratus* from Minnesota.
J. *A. pisi* isolated from pea and inoculated onto *Lathyrus odoratus.*
K. Potato-dextrose agar culture of *A. lathyri* isolated from pod of *L. odoratus,* Wisconsin.
L. *A. pisi* on *Lathyrus vernus* Germany (Oertel).
M. *A. lathyri* on pod of *L. odoratus,* Wis.
N. *A. lathyri* on everlasting pea (?), New Jersey.

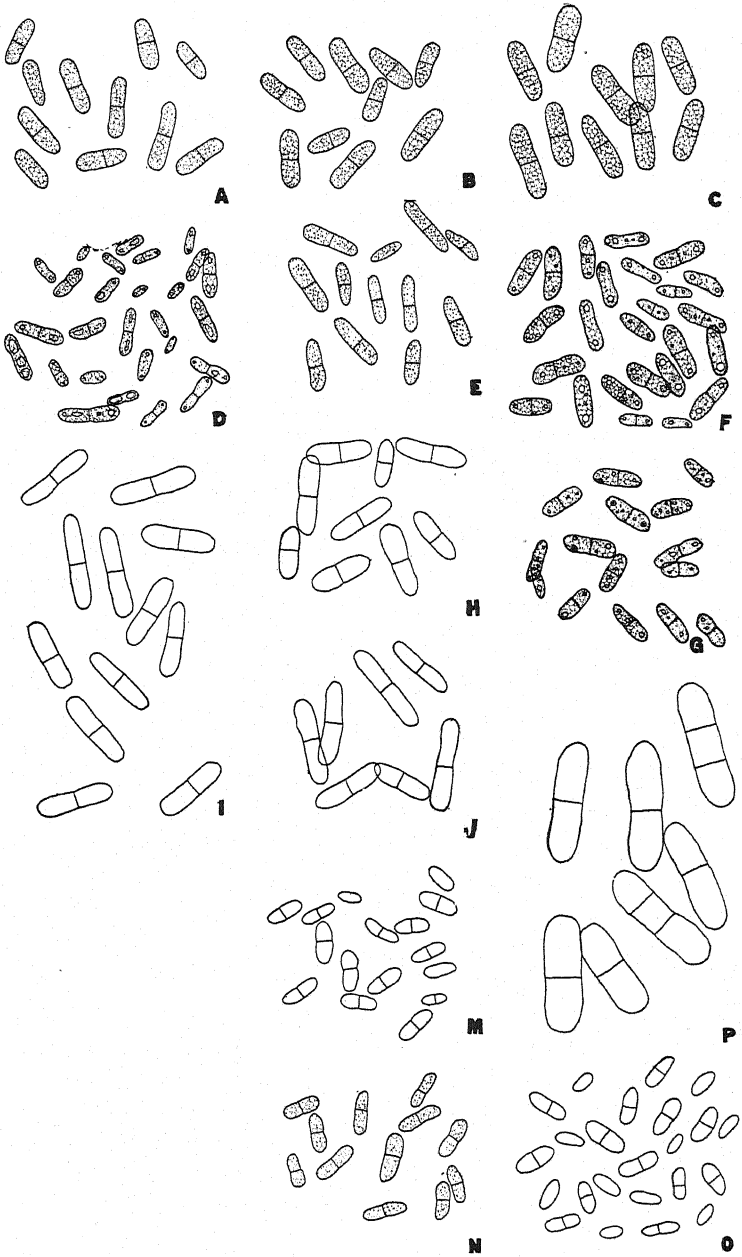
PLATE XXXVI

A. *Ascochyta imperfecta* from alfalfa. Leaf spot. Utah.
B. Same from Newtown, Ohio.
C. Same from Cincinnati, Ohio.
D. Isolation from Cincinnati, Ohio, *A. imperfecta* on potato-dextrose agar.
E. *A. imperfecta* on alfalfa. California material (Lee Bonar).
F. *A. pinodella* on pea. Leaf spot. Utah.
G. *A. pinodella,* leaf spot, Florida.
H. Ascochyta (*A. pisi* var. *medicaginis* Sacc.) on stems of alfala, Wendover, Wyoming. (Simmons.)
I. *A. medicaginis* on *Medicago lupulina.* Oxford, Ohio.
J. Same as fig. I. London, Ontario (Dearness.)
M. *A. phaseolorum* on *Phaseolus lunatus.* Takoma Park, D. C. Oct. 1908.
N. Same type as N. (Petrak Myc. Carpat. 402.)
O. Same as N. (Philippines, C. V. Piper.)
P. *A. boltshauseri* on *Phaseolus sp.* Albania (Petrak).



Pycnospore drawings from water mounts made with the aid of the camera lucida. ×1600. Spores with contents plasmolyzed through age or treatment are shown in outline only.


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MASKING OF SWEET-POTATO MOSAIC

L. L. HARTER AND W. A. WHITNEY

INTRODUCTION

A curious physiological phenomenon with respect to the mosaic of sweet potatoes (*Ipomoea batatas* Poir.) was accidentally discovered during the winter of 1926 and 1927 with plants growing in the greenhouse. In the fall of 1926 a number of roots from mosaic-infected Nancy Hall plants were bedded in a box which was located on a side bench above some steam pipes. The potatoes germinated normally and in about six weeks vines of considerable length had developed. The leaves were typically mosaic, being dark-green, deeply lobed, and distorted, and the surface covered with numerous white lines, closely resembling pencil marks, running indiscriminately in all directions. The foliage was so abnormal for the variety that one unfamiliar with it might easily have regarded it as a different variety.

It so happened that one of the vines accidentally grew over the side of the bench and hung in such a position that the steam, escaping in a considerable volume from a loose-fitting valve, not more than two feet distant, constantly flowed over it. While no records were made of the temperature, it was certainly very high, for it was uncomfortably warm in the vicinity of the vine. Exposed to high temperatures, the vine grew rapidly, and in a few days it was observed that the new leaves lacked some of the mosaic symptoms characteristic of the older leaves on the vine. The first leaves which unfolded under these conditions were not entirely normal when mature, neither were they typically mosaic. However, each succeeding leaf more closely approximated that of a healthy plant and more remotely that of the typical mosaic. During a period of about three weeks under these conditions the vine, about $2\frac{1}{2}$ feet long, bore apparently normal leaves at the distal 8 inches. The next 8 inches bore leaves with symptoms varying from typical mosaic to nearly normal leaves and the remainder of the vine, as well as all the several other branches and shoots, was typically mosaic.

In order to determine if the virus[1] causing the mosaic was absent from the portion of the vine bearing normal leaves, it was severed on the distal side of the first normal leaf about 6 inches back of the growing point, in order to remove only healthy tissue, and planted in a pot of sandy loam soil. The pot was placed in another part of the greenhouse where the temperatures ranged from 21° to 27° C. The cutting rooted and developed new growth which was typically mosaic, showing that the virus was not destroyed but only temporarily inactivated.

[1] The term "virus" as used in this paper is employed to designate the yet unknown causal principle.

TEMPERATURE AND HUMIDITY RELATIONS

From the behavior of this plant it was thought that temperature and humidity might be the controlling factors in the masking of mosaic symptoms. To test the validity of these suppositions an ordinary infection chamber, constructed of glass and located in a greenhouse, was equipped with a thermostatically-controlled heating unit, wet- and dry-bulb thermometers (for the determination of relative humidity), a pan of water, and a thermograph. A mosaic-infected plant (Fig. 1) was then placed in the chamber. The temperature fluctuated from 30° to 41.1° C. but averaged

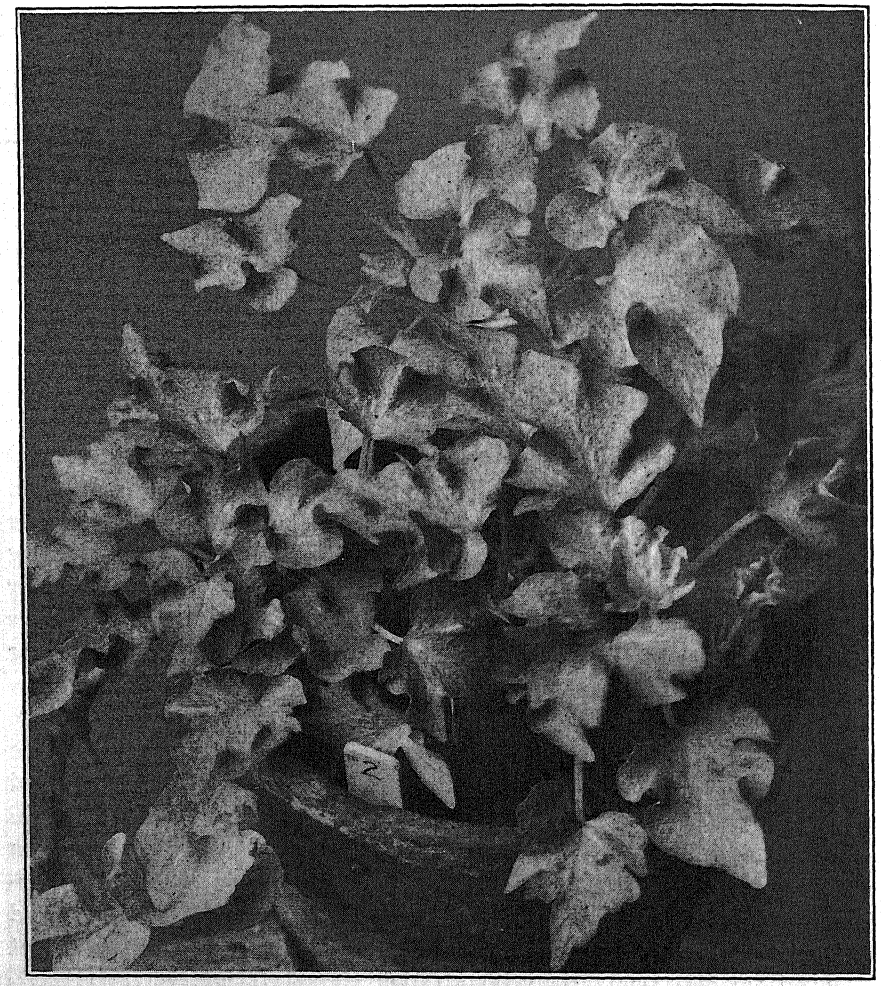


FIG. 1. A typically mosaic-infected plant used in one of the experiments. (Compare with Fig. 2.)

38.8° C., with an average relative humidity of 65.5 per cent. While sweet potatoes ordinarily will endure a high temperature, the leaves were unable during the winter to withstand the sudden shock of exposure to an extremely high temperature and gradually turned yellow and died. The roots and the older, hardier stems, survived, however, and after a period of acclimatization to the higher temperature, usually about four or five days, new branches and leaves developed. When the greenhouse became warmer with the approach of summer, the leaves did not die after being placed in the infection chamber to the same extent that they did during the winter and early spring. The new leaves appeared healthy in every respect. After the plant had developed a new growth of vines and leaves it was removed from the chamber and placed on a side bench where, in the course of a few weeks, other shoots (Fig. 2) developed, bearing leaves identical with those present when the plant was first placed in the chamber.

In these preliminary experiments an attempt was made to simulate the conditions that obtained when the steam flowed over the pendant branch. In order to study the effect of humidity and temperature on the masking of mosaic in more detail and under relatively-controlled conditions, two additional infection chambers were constructed. The chamber previously used, hereinafter designated as number one, was maintained as formerly; chamber two was equipped with thermostatic heat control alone; and chamber three was provided only with a pan of water to maintain a high relative humidity. Wet- and dry-bulb thermometers and a thermograph were placed in each chamber. Thus, in the chambers the effect of temperature and humidity, each acting alone and together, could be studied. Typical mosaic plants were selected, photographed, and one placed in each chamber. Readings of the wet and dry bulbs were made daily and water was added to the pots in sufficient amount to insure a normal growth.

The plant supplied with additional heat alone developed new shoots in about 4 or 5 days, and on the seventh day it was evident that the new foliage was typical of normal plants. The plant subjected to both additional heat and increased humidity developed new healthy shoots a few days later. Luxuriant growth was made by the plant in chamber 3, where the greenhouse temperature prevailed, and only the humidity was increased. The foliage of the third plant, while approximating the normal in appearance, differed from it in that the lobes were slightly deeper, somewhat malformed, and the veins lighter in color. In table 1 is summarized the temperature and humidity records taken during this experiment.

The plants were removed from the chambers at the end of 33 days and photographed, and then placed on a bench in the greenhouse where the tmperature was lower. In about a week the new growth showed typical symptoms of mosaic.

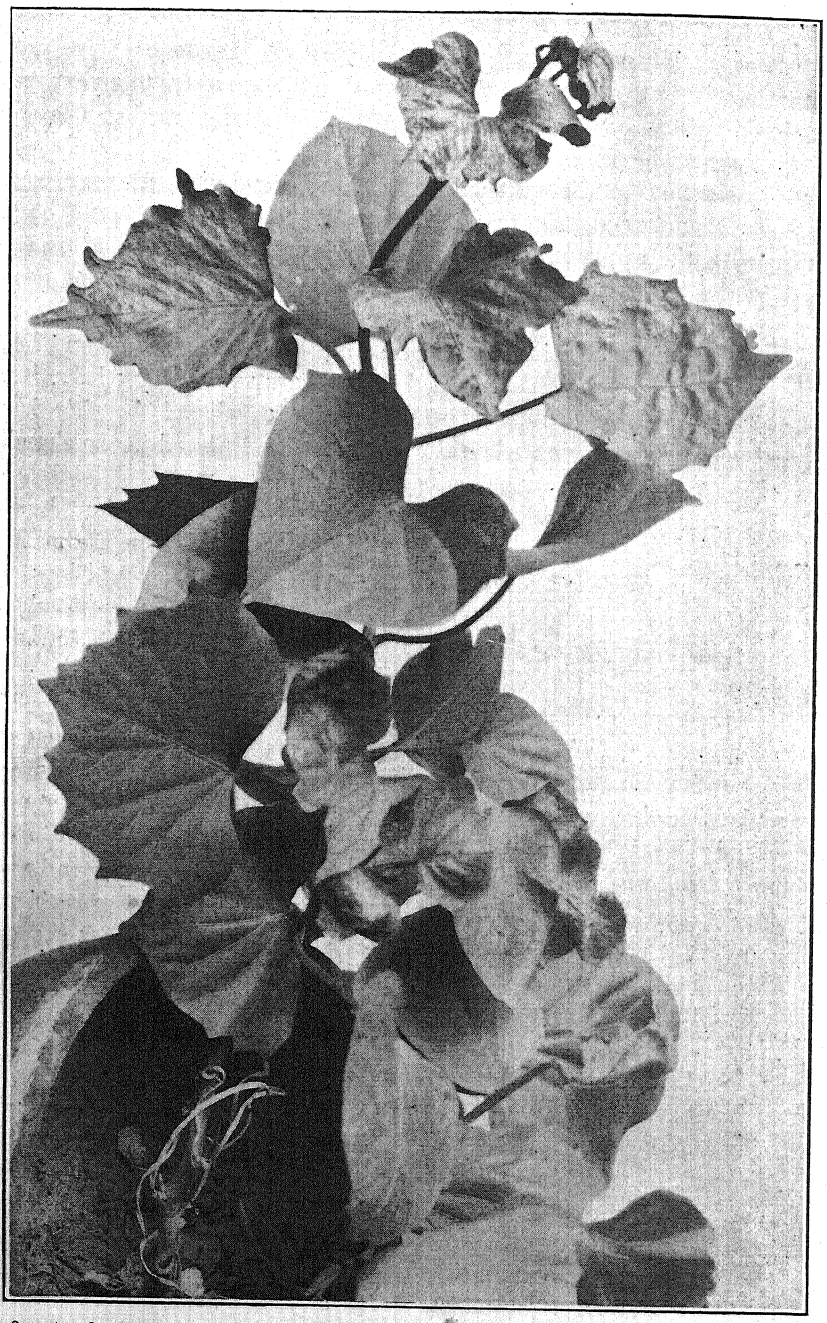


FIG. 2. A plant eleven days after removal from the chamber (37.8° C.). Note the typical mosaic symptoms on the new growth.

TABLE 1.—*Temperature and humidity in chambers 1 to 3*

Chamber No.	Treatment of Chamber	Temperature in °C.		Relative Humidity—Percentage	
		Range	Average	Range	Average
1	Temperature and humidity raised	35.0–42.7	40.8	46.0–95.9	72.5
2	Temperature only raised	33.3–45.6	38.3	44.0–85.0	59.3
3	Humidity only raised	22.8–40.0	32.8	79.5–100.0	92.0

These experiments were repeated several times with identical results.

EFFECT OF SOIL TEMPERATURE

In the above experiments the roots and aerial parts of the plant were subjected to the same temperature. There was, therefore, no evidence to show whether the change in the appearance of the foliage was due to the influence of the high temperature on the aerial parts of the plant alone, on the roots alone, or on both together.

Six constant-temperature tanks in a greenhouse were equipped with thermostatic heat control, and two each held at average temperatures of 39.8, 29.8, and 19.8° C. Each tank accommodated 8 pots which projected into the water 8 inches, thus subjecting 16 pots to each temperature. The plants were carefully selected and arranged so that there were 12 mosaic-infected and 4 normal ones at each temperature. The aerial parts of the plant were exposed to the greenhouse temperature which could not be controlled. The experiment, which lasted 31 days, was conducted in October and part of November at a time when the temperature of the greenhouse was not high at any time. A good growth was made at the two highest temperatures (39.8, 29.8° C.) and but little growth at the other (19.8° C.). Previous experiments showed that when the entire plant was subjected to a temperature approximately 40° C. mosaic was masked, the plant producing only normal-appearing foliage. The results of these experiments, however, show that a similar temperature about the roots had no effect on the foliage whatsoever, the new growth being in every instance typically mosaic.

AIR AND SOIL TEMPERATURE IN COMBINATION

Inasmuch as mosaic was not influenced by the temperature of the soil, the effect of various combinations of soil and air temperatures was studied, using two of the constant-temperature soil tanks described above. At the

outset one of these tanks (No. 1) was set to run at about 40° C. and the other (No. 2) at approximately 26° C. A glass chamber was placed over the front half of Tank No. 1 and the temperature therein adjusted to about 40° C. In each half of the tank was placed three mosaic-infected plants and one healthy one. Thus the four plants in the chamber were exposed to a soil and air temperature of 40° C. and the other four plants exposed to a soil temperature of 40° C. and to the air temperature of the greenhouse (about 23° C.). In Tank No. 2 the soil temperature was adjusted to about 26° C., a chamber placed over one half and a series of plants similar to that of Tank No. 1 set therein. In this tank half of the plants were subjected to a soil temperature of 26° C. and an air temperature of 40° C., the other half of the plants being exposed to a soil temperature of 26° C., with the air temperature that of the greenhouse.

A combination of air and soil temperature of 40° C. was too high, the plants dying in a few days. For this reason the water in Tank No. 1 and the air temperatures of the chambers over the two tanks were lowered to about 35° C. Plants exposed to these temperatures thrived both inside and outside of the chambers. The mosaic-infected plants within the chambers developed healthy-appearing foliage in about 10 days, while those outside continued to produce leaves infected with mosaic. The controls remained healthy in all cases. The experiment was repeated, the water in Tanks No. 1 and No. 2 being maintained near 35° and 25° C., respectively, and the chambers at an average temperature of 35° C. The experiment was terminated at the end of 45 days, with the result that none of the mosaic plants outside of the chambers showed any healthy growth, while normal foliage was produced by mosaic plants within the chambers, whether the soil temperature was 35° C. or 25° C.

DISCUSSION OF ENVIRONMENTAL RELATIONS AND MASKING

This reduction of mosaic symptoms may properly be classed as masking and, although rather well known for other mosaic diseases, it is the first time it has been reported for sweet-potato mosaic. It must not be overlooked that, due to our limited knowledge of the causes of mosaic diseases in general, no satisfactory explanation can be offered for this interesting phenomenon.

One of the earliest references to masking—then not so called—due to environmental conditions is that of Sturgis (8), who in 1899 observed that tobacco grown under shade is less severely attacked by mosaic than when grown in the open. Johnson (4), working with the same crop, showed that under controlled temperature conditions, the mosaic symptoms decreased above 30° C. and disappeared at 36–37° C. He (5) further showed that soy-bean mosaic was inhibited by temperatures of 26–28° C., slightly

higher than those for potatoes; that mosaic of pea beans resist higher temperatures; and that the mosaic diseases of clover and tomatoes (resulting from inoculation with tobacco mosaic) were inhibited by temperatures similar to those which masked mosaic of tobacco. Field observations of aster yellows and experimental work with mosaic of cucumbers led Doolittle (2) to conclude that environmental conditions affect the seasonal severity of these diseases, the degree being correlated with the vigor of the plants—the more vigorous the plant the shorter the mosaic incubation period. Elmer (3) came to the same conclusions, and stated that the appearance of mosaic is correlated with the vitality of the plant and not with any attenuation of the virulence of the virus involved. Tompkins (9) working with mosaic of potatoes concluded that air temperature is the only factor effective in masking and that only relatively short exposures at the critical temperatures—23–24° C.—are needed to mask the symptoms and also that the rate of masking was dependent on the length of exposure and the age of the leaves. On removal to a lower temperature the symptoms returned. Histological study showed that there was a difference between masked and normal leaves. Wilcox (10) observed that certain types of raspberry mosaic are masked during hot weather. Leaves that were mottled during cool weather remained so but the new growth was healthy.

That certain plants are carriers of mosaic is a theory advanced to explain the occurrence of mosaic diseases in crops where there is no apparent source of inoculum. *Physalis alkekengi* L. was shown by Nishimura (7) to carry the mosaic disease of tobacco, although itself exhibiting no external symptoms. *Nicotiana glauca* Graham may, according to Allard (1), show but slight symptoms of mosaic, yet the expressed juices be highly infectious to tobacco. Melhus (6) found that *Physalis longifolia* Nutt. carried mosaic over the winter and transmitted it to various solanaceous plants. He also showed that mosaic symptoms are masked on eggplants that have passed beyond the seedling stage.

The suppression of mosaic symptoms in the Nancy Hall sweet potato occurs when the plants are exposed to high temperatures whether or not in the presence of a high relative humidity. The most rapid and complete masking occurred in chambers with relatively low humidity and high temperature. In chambers with high temperature and high humidity masking took place more slowly. A partial masking, whose degree increased with an increase in temperature, occurred in the chambers with a variable temperature and high humidity. During the first part of one experiment, there was a period when the greenhouse was quite cool, especially during the night, and the temperature of the chamber with no added heat dropped quite low. Under these conditions practically no masking occurred, but with the resumption of warmer weather and the consequent rise in the

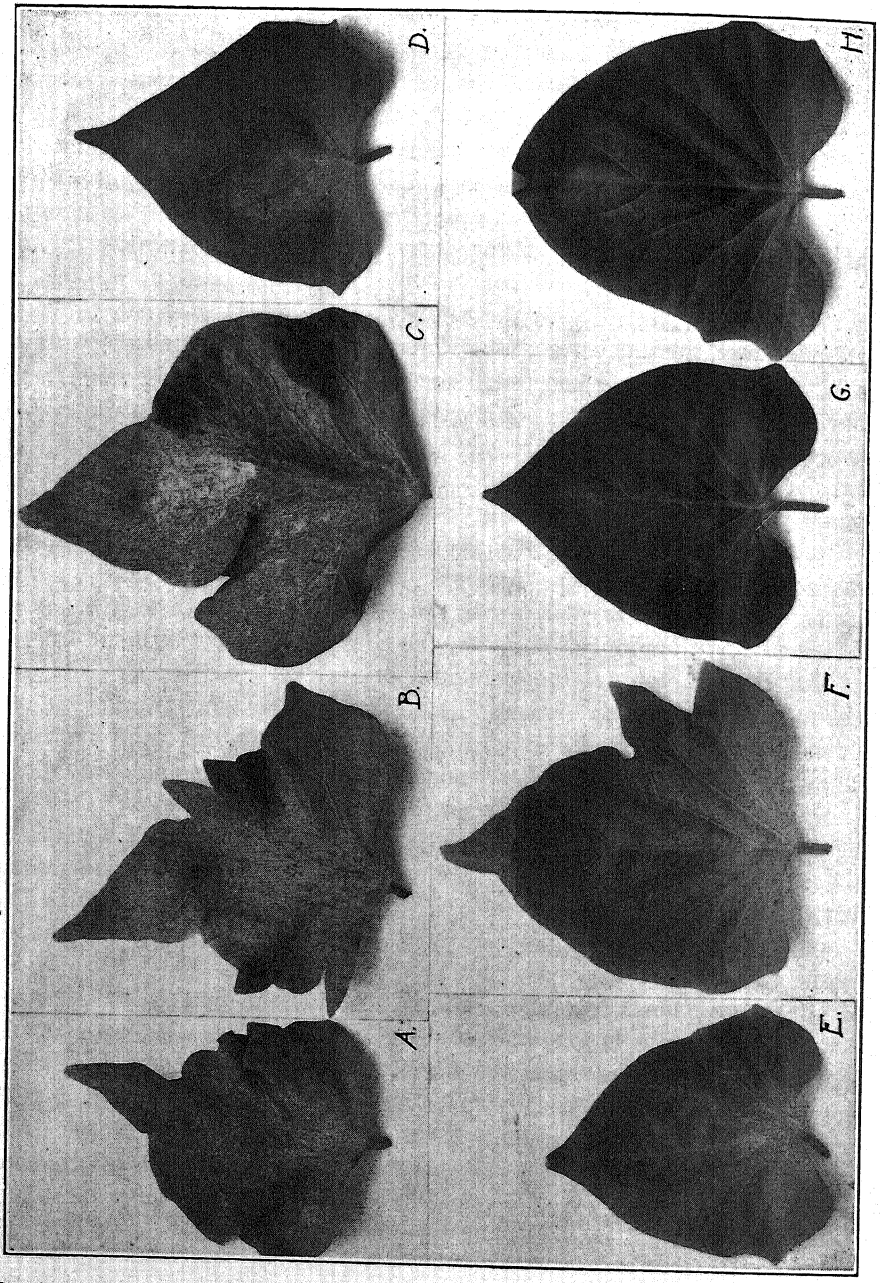


FIG. 3. A series of leaves showing the transition stages from mosaic to healthy leaves under the influence of an air and soil temperature of about 35° C. and an average air relative humidity of 87.5 per cent.

temperature of the chamber masking became apparent, although the degree was markedly less than in any one of the other chambers.

The first new leaves which develop under the influence of a high temperature are not fully normal, but each succeeding leaf more nearly approximates the healthy ones. Figure 3 shows a series of eight leaves and illustrates the various steps in the change from mosaic to healthy leaves. These leaves were grown under the influence of high temperature and taken in sequence from a single vine, with the exception of H which is from a healthy plant. An inspection of the illustrations shows considerable malformation in leaves A–C but with a tendency to normal shape and size. Leaves D and E are normal in all respects, except that there still remains some of the white marking at the base of the leaf. Leaf F is lobed on one side, while leaves G and H are both normal for the variety, leaf H having been healthy from the beginning. Mosaic leaves never change to normal leaves. Normal leaves on a mosaic plant never become mosaic, regardless of the conditions under which they are held.

It appears, as was found by Tompkins (9) with regard to potato mosaic, that temperature is the primary factor governing masking, with humidity playing a slight counteracting rôle. The exact range of temperatures at which masking takes place is not definitely known. It is safe to assume, however, that sweet potato mosaic falls into the high temperature group along with that of tobacco (4) and of clover (5).

SUMMARY

Masking of the mosaic symptoms of sweet potatoes (*Ipomoea batatas*) as a result of high temperature is reported.

The symptoms in the new growth are completely masked at a temperature of about 38° C. with or without an accompanying high humidity. A comparatively high relative humidity seems to retard rather than augment masking.

Mosaic symptoms recur on the new growth following the removal of the plants to lower temperatures. All changes take place on new growth alone; old growth, whether healthy or diseased, does not alter.

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THE EFFECT OF BORDEAUX MIXTURE ON THE INTERNAL TEMPERATURE OF POTATO LEAFLETS[1]

PAUL E. TILFORD AND CURTIS MAY

Several factors have been suggested as the cause of the physiological effect of Bordeaux mixture on potato plants. Among these are a stimulation by some of the ingredients of the spray, the alteration of the transpiration rate, an increase in the assimilatory capacity of the leaves, and the shading of the leaf by the Bordeaux film. No attempt will be made here to review this literature except in so far as it bears directly on the present problem.

Schander (13) measured the temperature of sprayed and unsprayed leaves of *Pachysphytum bracteosum* by placing a thermometer back of leaves exposed to the sun. He obtained no consistent differences. Lutman (10) concluded that potato leaves sprayed with Bordeaux mixture must be cooler than unsprayed leaves, since a thermometer held under a glass slide sprayed with Bordeaux mixture registered one-third to two-thirds of a degree Centigrade lower temperature than when exposed to direct sunlight.

According to Butler (2), the physiologic response resulting from Bordeaux mixture on potato plants, depends on the intensity of the shadow cast by the Bordeaux film.

The writers have been unable to find in the literature, thoroughly reviewed by Clum (3) (4), Ehlers (8), and also by Miller and Sanders (12), any accurate data on the influence of Bordeaux mixture on leaf temperature.

METHODS

The method of recording the leaf temperatures was essentially the same as that employed by Clum (3). Copper and Constantin thermocouples made of very small wire and equipped with a ten-foot extension were used.[2] A Leeds and Northrup type K potentiometer, type R galvanometer, Eppley standard cell, storage batteries, and a thermos bottle constituted the equipment. The thermocouples were calibrated by making voltage readings over a series of known temperatures. One junction was kept at a constant low temperature by inserting it in a thermos bottle filled with chipped ice and distilled water. The temperature of this cold bath was continually checked with a one-tenth-degree thermometer. The other junction was

[1] Published with the permission of the Director of the Ohio Agricultural Experiment Station.

[2] The thermocouples were purchased from the Cambride and Paul Instrument Company, Grand Central Terminal, New York, N. Y.

placed in water baths of known temperature. In making the readings the tip of the thermocouple was held as closely as possible to the bulb of a tenth-degree-thermometer and at the same instant that the voltage was read the thermometer reading also was noted. By plotting the voltages as abscissas and temperatures as ordinates a straight line was secured from which voltage readings could readily be converted to temperature readings. The wires directly back of the tip of the couple were reinforced with De Kotinsky cement. This made it possible to insert the point into a leaf without danger of breaking the fine wires.

The instruments were set on a solid table in the greenhouse and protected from the sun by heavy burlap curtains. Uniform potato plants, 15–18 inches high, were used. The upper and lower surfaces of the leaves of two plants were sprayed with one material and of two similar plants with another. They were well watered an hour before readings were made so the leaves would be turgid. When readings were to be made one unsprayed and one sprayed plant, or two plants sprayed with different materials, were set side by side on a box in full sunlight. The thermocouple was inserted into the lower side of the midrib of a leaflet which was then held about perpendicular to the sun's rays. As soon as the voltage became stable the reading was made. The thermocouple was then inserted into a leaflet of the second plant and a reading made. It was found that readings could not be made in less than one to one-and-a-half minutes because, immediately after inserting the thermocouple, the voltage was too high. In the course of a minute to a minute-and-a-half the voltage dropped to a lower and stable point. This effect was due probably to the wounding of the tissue when the couple was inserted.

The couple was inserted in the midrib because the mesophyll of the lamina of potato leaflets is so thin that the couple could not be placed between the upper and the lower epidermis. Smith (15), however, has shown that readings taken in the midrib are comparable with those taken in the lamina of leaves.

Ten readings were made on each plant. The first pair of plants was then replaced by a second pair and a similar set of readings taken, making twenty determinations for each comparison. Sometimes, but not often, more than one or two readings were made on a single leaflet. All the determinations were made during February and March in the greenhouse.

RESULTS

Results were obtained comparing the temperature of leaves of plants sprayed with 4–4–50 Bordeaux and unsprayed leaves during both cloudy and sunny weather; 4–4–50 and 4–6–50 (hydrated lime) and 4–4–50 and 20–80 copper lime dust. These data are given in table 1.

TABLE 1.—*Comparison of the cooling effect of different Bordeaux mixtures and copper-lime dust on the internal temperature of potato leaves*

Shade—Cloudy weather			Sun shining brightly								
4–4–50	No spray	Diff. in favor of 4–4–50	4–4–50	No spray	Diff. in favor of 4–4–50	4–6–50	4–4–50	Diff. in favor of 4–6–50	4–4–50	20–80 dust	Diff. in favor of 4–4–50
°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
20.0	20.1	−0.1	25.6	27.7	−2.1	26.1	25.8	+0.3	30.1	29.3	+0.8
20.6	20.6	0.0	26.6	27.9	−1.3	26.4	32.9	−6.5	29.6	28.7	+0.9
20.6	21.3	−0.7	23.9	25.6	−1.7	26.4	26.1	+0.3	29.5	31.2	−1.7
21.2	21.8	−0.6	24.3	26.3	−2.0	27.7	28.2	−0.5	26.3	27.5	−1.2
20.8	21.1	−0.3	24.0	25.0	−1.0	24.3	27.1	−2.8	28.5	28.2	+0.3
19.2	19.2	0.0	24.5	24.8	−0.3	26.3	25.7	+0.6	28.6	27.8	+0.8
18.9	18.9	0.0	24.9	24.7	+0.2	27.3	27.0	+0.3	27.9	28.2	−0.3
19.2	18.9	+0.3	24.5	25.7	−1.2	26.2	27.9	−1.7	27.6	26.8	+0.8
19.5	18.7	+0.8	25.8	24.5	+1.3	26.5	26.2	+0.3	29.3	28.0	+1.3
19.5	20.0	−0.5	21.3	24.3	−3.0	27.0	29.5	−2.5	29.2	27.8	+1.4
19.8	19.9	−0.1	23.0	25.6	−2.6	30.5	30.4	+0.1	26.1	27.9	−1.8
22.1	23.7	−1.6	23.7	25.6	−1.9	28.8	28.3	+0.5	25.2	26.9	−1.7
22.4	23.7	−1.3	29.1	29.1	0.0	28.5	28.9	−0.4	25.5	25.8	−0.3
22.0	23.1	−1.0	25.9	30.0	−4.1	28.7	29.4	−0.7	24.6	26.5	+0.1
22.7	21.1	+1.6	25.4	26.0	−0.6	29.7	31.1	−1.4	24.9	26.5	−1.6
22.9	22.9	0.0	26.4	28.5	−2.1	29.3	29.6	−0.3	24.9	26.1	−1.2
23.1	22.2	+0.9	27.8	29.3	−1.5	27.8	30.1	−2.3	26.3	26.4	−0.1
22.8	22.0	+0.8	28.0	29.0	−1.0	29.7	30.0	−0.3	25.3	25.6	−0.3
23.7	24.2	−0.5	26.6	27.9	−1.3	29.8	30.2	−0.4	26.6	25.9	+0.7
23.7	22.5	+1.2	24.8	27.7	−2.9	29.0	30.9	−1.9	25.6	25.6	0.0
Average difference		−0.055			−1.45			−0.96			−0.15
Odds		1:1		Greater than	10000:1			113:1			2.6:1

Leaves sprayed with Bordeaux plus 1 per cent lamp black were compared with unsprayed leaves; in addition, 4–4–50 Bordeaux was compared with a lime spray (6 lb. to 50 gal. water). Finally, measurements also were made on the leaves of unsprayed plants to determine what variability might be expected. These data are given in table 2.

TABLE 2.—*Comparison of the internal leaf temperatures of potato plants treated in different ways*

No. 1 No spray	No. 2 No spray	Diff. in favor of No. 2	No spray	4–4–50 plus 1 per cent lamp black	Diff. in favor of no spray	0–6–50 lime	4–4–50	Diff. in favor of lime
°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.	°C.
27.4	27.7	+0.3	27.1	34.3	−7.2	24.3	24.1	+0.2
27.8	27.2	−0.6	32.0	37.4	−5.4	24.7	25.4	−0.7
27.2	28.4	+1.2	29.7	34.6	−4.9	26.4	26.8	−0.4
28.7	27.9	−0.8	31.7	32.7	−1.0	26.3	28.3	−2.0
30.6	29.8	−0.8	29.9	34.2	−4.3	26.8	27.1	−0.3
30.9	33.4	+2.5	28.8	33.0	−4.2	28.2	28.0	+0.2
34.3	32.8	−1.5	27.5	30.7	−3.2	26.9	28.3	−1.4
32.9	31.6	−1.3	27.3	29.2	−1.9	26.0	27.2	−1.2
31.1	31.1	0.0	28.4	33.4	−5.0	27.0	28.6	−1.6
30.9	31.4	+0.5	31.3	36.9	−5.6	27.9	28.8	−0.9
31.9	32.4	+0.5	32.7	33.2	−0.5	25.9	28.0	−2.1
31.8	32.4	+0.6	33.2	34.2	−1.0	28.9	27.0	+1.9
32.2	31.6	−0.6	31.1	33.3	−2.2	27.8	29.8	−2.0
33.9	32.2	−1.7	31.7	34.6	−2.9	29.7	31.2	−1.5
34.4	36.3	+1.9	31.7	33.0	−1.3	28.8	28.8	0.0
33.1	33.3	+0.2	31.0	33.0	−2.0	26.8	27.8	−1.0
33.0	33.0	0.0	32.1	34.1	−2.0	28.2	30.9	−2.7
30.7	31.4	+0.7	32.0	33.0	−1.0	28.2	28.7	−0.5
30.6	31.3	+0.7	30.3	32.0	−1.7	28.9	30.9	−2.0
33.8	31.4	−2.4	30.7	31.9	−1.2	27.4	28.0	−0.6
Av. Difference		−0.025			−2.92			−0.93
Odds		1.18:1	Greater than		10000:1			4999:1

At first sight the readings may appear more variable than one would expect. However, when the factors causing this variability are understood, the results are as consistent as could be hoped for. Changes in light intensity, air-convection currents, the angle of incidence of the sun's rays, the age of the leaf, length of exposure to direct sun rays, and the state of turgidity of the leaves have been pointed out by Eaton and Belden (7) as factors influencing rapid changes and variations in leaf temperatures. Variations in the thickness of the spray coating undoubtedly account for

some of the variations obtained in the results here reported. While every possible precaution was taken to minimize these factors or, at least, to have them comparable throughout a series of readings, yet slight variations occurred. Clum (4) reports similar experiences. When he placed thermocouples in the right and left sides of a cabbage leaf, and then placed the leaf so that both sides were exposed to the sun approximately alike, he found that in two-thirds of the readings the temperatures on opposite sides of the leaf varied about one degree Centigrade. Consequently, several readings are necessary to obtain significant results.

It was possible to calculate "t" for the mean in each case, since the readings were taken in pairs. The odds that the results are significant were obtained from the revised tables of "t" by Student (16). It is clearly evident from the data presented that 4–4–50 Bordeaux mixture has a cooling effect on leaves when exposed to the sun. This is not true, however, in cloudy weather. Leaves sprayed with 4–6–50 were significantly cooler than those sprayed with 4–4–50 Bordeaux. Also, leaves sprayed with lime suspension, 6 pounds to 50 gallons, were cooler than leaves coated with 4–4–50 Bordeaux. Copper-lime dust produced the same cooling effect as 4–4–50 Bordeaux. Leaves sprayed with Bordeaux plus one 1-per-cent lamp black were decidedly hotter than unsprayed leaves.

DISCUSSION

It is not surprising that leaves sprayed with Bordeaux mixture should be cooler than unsprayed leaves. The film of spray gives the leaf a whitish cast which reflects the light rays, thus acting as a shade over the sprayed leaf. That the whitest Bordeaux reflects the most light is evidenced by the fact that leaves sprayed with 4–6–50 Bordeaux, and leaves sprayed with lime alone were cooler than leaves sprayed with 4–4–50. This, perhaps, explains the benefits sometimes derived from adding extra lime to Bordeaux mixture for potato spraying during July and August. It might also be said that the lamp-black spray shades the leaf, but, in this case, the light rays are absorbed and, as a result, the leaves are hotter than unsprayed leaves.

The fact that potato leaves sprayed with Bordeaux mixture are cooler than unsprayed leaves may partly explain the benefits derived from spraying in certain localities during years when diseases and insects are not factors. Although the lowering of the leaf temperatures as reported in the experiments is not large it might be considerably greater outdoors during the summer when the sun's rays are more intense. Even a reduction of 1.45° C. should have a beneficial effect in regions where the average temperature is above the optimum for potatoes. Bushnell (1) found that a rise in temperature from 20° C. to 23° C. reduced the weight of tubers

per plant from 20.9 grams to 5.0 grams. If it is true that the lowering of the leaf temperature is beneficial to the potato, in sections where the average temperature is above the optimum for the potato, it is also probably true that, in regions where the average temperature is near the optimum, no beneficial effects would be manifest. This may explain why no benefits result from spraying in the absence of disease in certain sections of Europe, as reported by Lutman (10), and in the Upper Peninsula of Michigan, as stated by Kotila and Coons (9).

Since sprayed leaves are cooler than unsprayed leaves, one would expect their transpiration rates would also be lower. Such, however, apparently is not the case, since Duggar and Cooley (5), Duggar and Bonns (6), Martin (11), and Shive and Martin (14) have shown that, in general, plants sprayed with Bordeaux have a higher transpiration rate than unsprayed plants. In the paper of Duggar and Bonns it is pointed out that this increase in transpiration occurs during the night interval and that during bright, sunshiny days the sprayed and unsprayed potato plants transpire about the same. In view of the fact that Clum (3) was unable to correlate small variations in leaf temperature with corresponding variations in transpiration, it is not surprising that the sprayed leaves, although cooler, do not have a corresponding lower transpiration rate.

SUMMARY

1. A study of the effect of copper sprays and dusts on the internal temperature of potato leaflets has been made.

2. Bordeaux mixture and copper-lime dust have a slight cooling effect on potato leaflets.

3. The whiter the coating of Bordeaux, the greater is the cooling effect. Leaves sprayed with 4–6–50 Bordeaux or with lime suspension are cooler than leaves sprayed with 4–4–50.

4. A dark colored spray increased the internal temperature of sprayed leaflets.

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THE VALUE OF PHYSIOLOGIC-FORM SURVEYS IN THE STUDY OF THE EPIDEMIOLOGY OF STEM RUST[1]

E. C. STAKMAN, M. N. LEVINE, AND J. M. WALLACE

In the spring of 1917, following the terrific stem-rust epidemic of the previous season in the hard-red-spring-wheat region of the United States and Canada, the Office of Cereal Crops and Diseases of the United States Department of Agriculture, in cooperation with several State experiment stations, began a comprehensive study of the epidemiology of cereal rusts, especially stem rust, *Puccinia graminis* Pers. One of the principal objects was to determine the sources of stem rust in the spring in the spring-wheat region of the upper Mississippi Valley, where the disease does the greatest damage.

It was considered that there were three possible sources of stem rust in the spring: 1, urediniospores which survived the winter on fall-sown grains and on grasses within the region; 2, aeciospores from barberry bushes; 3, wind-borne urediniospores from the South. From extensive experiments and observations it has been found that in the northern grain-growing States the uredinial stage of the rust does not persist from one crop season to the next except occasionally under unusually favorable conditions in limited areas.[2] For practical purposes this source of rust can be disregarded. As early as 1917 it became perfectly evident, on the other hand, that in the spring-wheat region much, if not most, of the rust in the spring came from barberry bushes (4, 7). The question remained whether the barberry was solely responsible for the development of epidemics or whether the uredinial stage could survive the winter in the southern United States and in the spring produce large numbers of urediniospores which might be blown northward in early summer and cause widespread infection in the spring wheat area.

As a result of overwintering studies, it has been shown that the uredinial stage of stem rust usually persists through the winter in the general district around San Antonio, Texas, but seldom so far north as Dallas, Texas (1). By the middle of May the uredinial stage of the rust usually is abundant

[1] Cooperative investigations between the Office of Cereal Crops and Diseases, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station. Published with the approval of the Director as Paper No. 848 of the journal series of the Minnesota Agricultural Experiment Station.

[2] The general statements in this paper, unless otherwise specified, are based on personal observations and detailed experiments made in connection with the general rust-epidemiology study. Most of the results have not yet been published in detail, but they are available in summarized reports on file in the Office of Cereal Crops and Diseases and at the Minnesota Agricultural Experiment Station.

enough in the wheat-growing portions of Texas to furnish a large amount of potential inoculum for the grain fields of the North. Therefore attempts were made to ascertain whether these southern urediniospores could be blown northward early enough in the season and in sufficient quantity to contribute significantly to the development of epidemics.

Three principal methods were used in attempting to answer this question. Direct observations were made on the progressive development of stem rust from Texas northward in the spring and early summer; spore traps were exposed near the ground, from high buildings, and from airplanes and balloons, in order to obtain evidence regarding the number of spores in the air in regions where rust had not yet appeared (5); and physiologic-form surveys of *Puccinia graminis tritici* were made each year in order to find out whether the forms in the South were the same as those in the North. If the forms in the South were the same as those farther north, there would be circumstantial evidence that the rust might have been blown northward. On the other hand, if the forms in the South were distinctly different from those in the North, there would be presumptive evidence that the epidemics in the North could not have resulted from spores blown northward by the wind.

Final conclusions could not be drawn from the direct observations on the progressive development of rust from south to north. In some seasons rust in the North appeared to come almost solely from barberry bushes, whereas in other seasons there was evidence of migration of urediniospores from south to north. The migration apparently was gradual in some seasons and sudden in others. It seemed that in some seasons the urediniospores probably were disseminated relatively short distances from areas where rust was prevalent, and the northward movement appeared to be gradual. In other years the urediniospores seemed to be disseminated over a wide area in a short time. For example, in 1925, strong winds originating in Mexico, Texas, and Oklahoma blew northward almost continuously for about a week early in June. Within about a week after the first rains following the wind there was wide-spread and rather uniform infection of wheat in the spring-wheat area. This was strong circumstantial evidence that the south winds had disseminated urediniospores over a wide area from Texas to the Canadian boundary or beyond. But in some years *P. graminis avenae* seemed to have migrated from the South to the North, while *P. graminis tritici* did not; in other years the reverse was true. And even in years when both seemed to migrate, there was no evidence that the urediniospores of *P. glumarum* (Schm.) Eriks. and Henn. and of *P. coronata* Corda were blown northward, except for the latter in 1927.

It was decided, therefore, to intensify physiologic form surveys which had been made each year since 1917, because of their value in the problem

of breeding rust-resistant varieties and in predicting the probable efficacy of the barberry eradication campaign as a rust control measure.[3]

The results obtained soon explained why certain varieties of wheat sometimes were resistant in one locality and susceptible in another in the same year, or resistant in the same locality in some years and susceptible in others. In 1917 and 1918 H. D. Barker and the senior writer observed that several varieties of hard spring wheat, quite susceptible in the hard spring-wheat region, were highly resistant to the forms of *P. graminis tritici* which appeared to be most prevalent in the Southern States. For example, Marquis, C. I. 3641, Haynes Bluestem, C. I. 2874, and Glyndon, Minn. 163, remained free from stem rust when grown at Auburn, Ala., Bay Minette, Ala., and Baton Rouge, La., whereas certain other varieties, such as Alabama Bluestem, became heavily rusted when grown in neighboring plots. It was shown clearly by inoculation experiments in the greenhouse that this apparent immunity of the hard spring wheats was due merely to the absence of forms of *P. graminis tritici* which could infect them normally. For about two years the writers found no forms in the Gulf States which attacked the hard spring wheats normally. For example, in the spring of 1917 collections were made at Baton Rouge, La., Brundige, Ala., and Beaumont, Tex. All of them proved to be what was then known as *P. graminis tritici-compacti,* distinguished from ordinary *P. graminis tritici* primarily by its inability to infect hard spring wheats normally. Stakman and Hoerner (6, p. 148) made the following statement regarding this so-called form of rust, which in reality probably comprised several of the physiologic forms of *P. graminis tritici* subsequently described by Stakman and Levine (8) and now designated by numbers:

"The factors limiting the distribution of the rust and its possible importance in rust epiphytology are not known. It is possible that it is common only where soft wheats are grown, or where climatic factors may determine its geographic limits. If it proves to be wide-spread in the South it certainly renders less probable the hypothesis, held by many, of the seasonal spread of wheat stem-rust from south to north. It may also explain the fact that varieties of wheat show varying resistance in different regions. The whole question of the degree of constancy in the relations between the rust and its host plants is being investigated in connection with the rust epiphytology studies now being made by the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture."

[3] Physiologic form surveys have been made of *P. graminis avenae* also, but the results did not aid directly in the solution of the present problem, and therefore are not included in this paper. Similar surveys have been made since 1919 by the Dominion Rust Research Laboratory of Canada (2).

In 1918 it was found, however, that there were forms of *P. graminis tritici* in the South which could infect the hard spring wheats heavily, although it still seemed that those that could not so infect probably were more prevalent. Investigations therefore were made to ascertain whether the urediniospores of these forms were blown northward in the spring in sufficient numbers to cause epidemics in the spring-wheat region. The area in which the most intensive survey was made comprises Texas, Oklahoma, Kansas, Nebraska, Missouri, Iowa, Minnesota, South Dakota, and North Dakota.

The results obtained for several years were somewhat inconclusive, but those obtained in 1926, 1927, and 1928 seem significant and therefore are presented here in a preliminary way. A more detailed presentation of results will be made at a later time. The data are given in table 1 and figures 1 and 2.

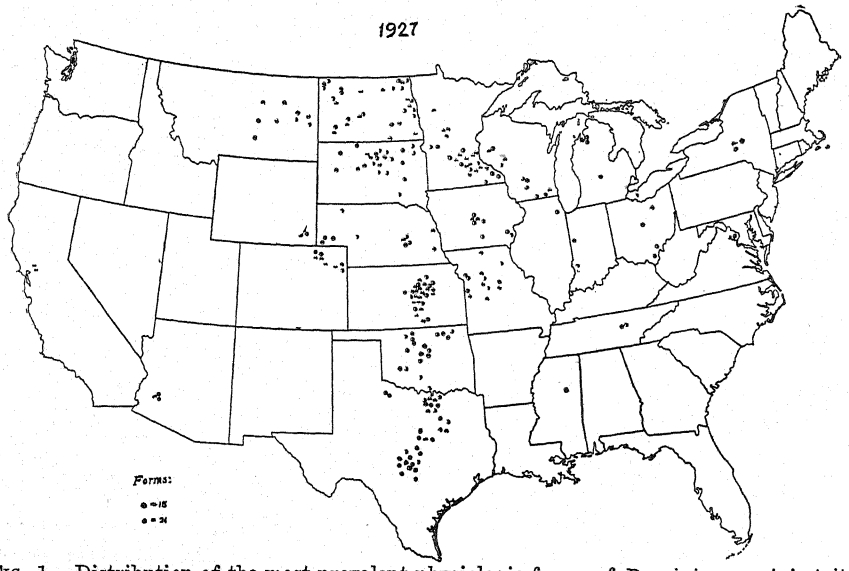


FIG. 1. Distribution of the most prevalent physiologic forms of *Puccinia graminis tritici* (forms 18 and 21) isolated in 1927.

It will be seen from table 1 and figure 2 that form 11 was fairly common in Texas in 1926, but it was not found in any of the spring-wheat States. Peltier and Thiel (3) state, however, that 13 of the collections which they made in Nebraska that year proved to be form 11. Possibly, therefore, form 11 migrated as far north as Nebraska but not so far as the spring-wheat area, although we may of course have missed it in our survey of the spring-wheat area. It also is possible that this form may have overwintered in the telial stage near barberry bushes in Nebraska and that urediniospores were not blown into the State from the South. This

TABLE 1.—*Distribution and prevalence of physiologic forms of Puccinia graminis tritici as indicated by the number of collections of each of several forms made in the Mississippi Valley in 1926 and 1927, and in Mexico and the Mississippi Valley in 1928*

Distribution	Physiologic forms by number																					Total number of	
	1	2	3	11	17	18	19	21	23	28	29	32	33	34	36	38	39	49	50	51	52	56	Cultures	Forms
1926																								
Texas	—	—	—	6	1	1	1	2	—	—	—	—	—	—	2	—	4	—	—	—	—	—	17	7
Oklahoma	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	2
Kansas	—	—	—	—	—	1	—	1	—	—	—	—	—	1	2	—	—	—	—	—	—	—	5	4
Nebraska	—	—	—	—	—	2	—	2	—	—	—	—	—	—	4	—	—	—	—	—	—	—	8	3
Missouri	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1
Iowa	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1
South Dakota	—	—	—	—	—	2	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	4	3
North Dakota	—	—	—	—	—	2	—	2	—	—	—	—	—	—	2	—	—	—	—	—	—	—	6	3
Minnesota	—	—	1	—	—	10	—	1	—	—	—	2	1	—	5	1	—	—	—	1	—	—	22	8
Total	—	—	2	6	2	21	1	8	—	—	—	3	1	1	16	1	4	—	—	1	—	—	67	13
1927																								
Texas	1	—	—	7	3	12	2	20	—	1	—	—	—	—	2	3	1	—	—	—	—	—	52	10
Oklahoma	2	—	—	—	1	8	—	10	—	—	—	1	—	—	2	1	2	1	—	—	—	—	28	9
Kansas	1	—	—	3	—	18	2	9	—	—	—	1	—	—	1	—	7	1	—	—	—	—	43	9
Nebraska	—	—	—	—	3	6	1	5	—	—	—	—	—	—	3	—	1	1	—	—	—	—	20	7
Missouri	—	—	—	1	1	7	—	6	—	—	—	—	—	—	—	—	2	1	—	—	—	—	18	6
Iowa	—	—	—	1	—	2	—	6	—	—	—	—	—	—	3	1	—	—	—	—	—	—	13	5
South Dakota	—	—	—	—	4	9	—	14	—	—	—	1	—	—	1	1	2	—	—	—	—	—	32	7
North Dakota	—	—	—	1	—	14	—	16	—	—	—	—	—	—	2	—	—	—	—	—	—	—	33	4
Minnesota	—	—	—	—	2	15	1	10	—	—	—	3	—	—	6	—	1	2	—	—	—	—	40	8
Total	4	—	—	13	14	91	6	96	—	1	—	6	—	—	20	6	16	6	—	—	—	—	279	12
1928																								
Mexico	—	—	—	1	5	—	—	2	3	—	—	1	—	—	1	39	2	5	1	—	1	—	61	11
Texas	—	—	—	2	—	—	1	7	—	—	1	2	—	—	5	20	—	4	—	—	—	—	42	8
Oklahoma	—	—	—	—	—	1	2	2	—	—	—	1	—	—	3	8	—	4	—	—	—	—	21	7
Kansas	1	—	—	5	—	3	2	9	—	—	—	—	—	—	10	2	4	3	—	—	—	—	39	9
Nebraska	—	—	—	—	1	—	—	8	—	—	—	4	—	—	8	4	3	1	—	—	—	—	29	7
Missouri	—	—	—	—	1	—	—	1	—	—	—	1	—	—	5	—	—	1	—	—	—	—	9	5
Iowa	1	—	—	—	2	1	—	4	—	—	1	—	—	—	8	1	4	1	—	—	1	2	26	11
South Dakota	—	—	—	—	—	1	1	9	—	—	—	—	—	1	16	2	1	1	—	—	—	—	32	8
North Dakota	1	—	—	—	2	—	—	3	—	—	—	1	—	—	8	1	—	1	—	—	—	—	17	7
Minnesota	2	1	—	—	1	1	1	15	—	—	1	1	—	—	18	1	—	5	—	—	1	—	48	12
Total	5	1	—	8	12	7	7	60	3	—	3	11	—	1	82	78	14	26	1	—	3	2	324	20

explanation seems plausible in view of the fact that Peltier and Thiel obtained some of their collections of form 11 near barberry bushes. It may be possible also that some of the collections identified by Peltier and Thiel

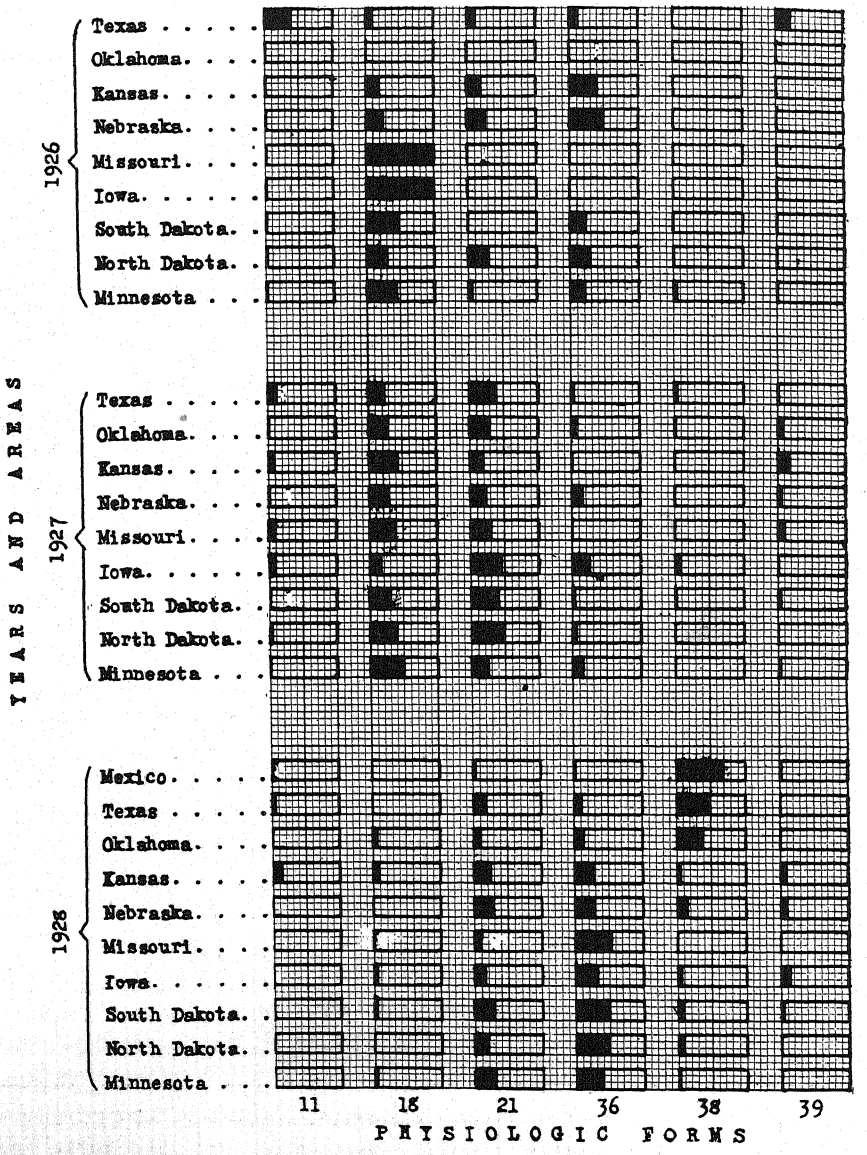


FIG. 2. Proportional distribution (per cent of total number of isolations in each case) of the most prevalent physiologic forms of *Puccinia graminis tritici* in the Mississippi Valley in the years 1926, 1927, and 1928.

as form 11 may have been form 32, as the two differ only in minor respects which are not always apparent. Form 11 produces a type 4 infection on the durum varieties used as differential hosts, whereas form 32 produces a type x infection, which may appear like type 4 unless environmental conditions are very favorable for the development of the type x infection. Unless several series of inoculations are made, therefore, form 32 might quite naturally be identified as form 11.

In any case, the most prevalent forms in the North were clearly 18 and 36, whereas those in Texas were 11 and 39. If these results can be relied upon, therefore—and the writers think they can—it is very unlikely that much rust was blown northward, because form 11 attacks the hard spring wheats and the durums heavily and there is no known reason why it should not have developed abundantly if it had made its way into the Northern States.

The results in 1927 were quite different from those in 1926. In 1927 a large number of collections were identified, and forms 18 and 21 clearly were the most outstandingly prevalent. As they appeared to be widely distributed from Texas northward, there is nothing in the results of the survey which would indicate that rust might not have been blown northward in sufficient quantity to account, at least in part, for the rust epidemic in the North.

The 1928 results are perhaps the most interesting of all. According to unpublished studies made by Wallace Butler, the uredinial stage of stem rust of wheat did not survive the winter in abundance even in the San Antonio district of Texas during the winter of 1927–28. Furthermore, the nature of the primary infection in Texas in the spring indicated that the rust had been blown into Texas from farther south. The most probable source, of course, would be Mexico. Collections were made in Mexico, therefore, as well as in the States from Texas northward. Of 61 Mexican collections, 39 proved to be form 38, and 3 proved to be form 23. Form 23 was not found at all in the North, but form 38 was quite abundant in Texas and Oklahoma. It also was found a few times farther north. Probably, therefore, this form of rust may have been blown from Mexico into Texas and Oklahoma and thence northward. But form 38 produces only very weak infection on the hard spring wheats, although it produces a type x infection on the durums. Had the only source of rust in the spring-wheat region, therefore, been that in the South, it seems very improbable that an epidemic could have developed even had weather conditions been exceptionally favorable. As a matter of actual fact there was no wide-spread epidemic of stem rust on wheat in the spring-wheat region in 1928. Whether this was because of the relatively small amount of effective inoculum which

came from the South or because of unfavorable weather conditions for rust development in the North, can not be stated with certainty. It seems significant, however, that in this year the form of rust which was by far the most abundant in the far South was relatively innocuous as far as the wheats in the spring-wheat region were concerned.

The results of the survey in 1928 suggest the possibility that physiologic-form surveys may be used in the future not only to explain certain facts in connection with the development of epidemics, as they already have done, but also to predict the probable occurrence of epidemics. When most of the barberries have been eradicated from the spring-wheat region, there probably will be only one important source of inoculum in the spring, namely, the urediniospores in Mexico and Texas.[4] The rust ordinarily develops fairly abundantly in Mexico during March and April, and by May it usually is abundant in Texas. It is entirely possible, then, that surveys could be made to determine which physiologic forms are present in the far South in time to be of some value in predicting the probability of epidemics in the North. Other factors besides the virulence of physiologic forms are important in governing the development of rust epidemics, but certainly if the forms of rust in the South in any given year are predominantly those which can not attack the wheats grown in the North, there would be little to fear even though the weather conditions were favorable for epidemics. On the other hand, if the forms of rust in the South were such as could attack the spring wheats normally, there would still be no way of predicting the likelihood of epidemics. It could merely be stated that an epidemic might occur if there were strong and long-prevalent south winds and if weather conditions were especially favorable for the development of rust in the North.

The results of the surveys thus far made show that the prevalence of physiologic forms in certain regions may vary greatly from year to year. In so far as these surveys bear on the question of the source of rust in the spring-wheat region, the following conclusions are justified. In some years the same physiologic forms seem to predominate throughout the wheat-growing region of the Mississippi basin from Texas to the Canadian border. This was true in 1927, and in some previous years. In 1926, however, the form of rust most prevalent in Texas did not make its appearance in the hard spring-wheat area. Therefore, it is unlikely that there could have been an important south-to-north migration of rust. In 1928 the rust may have been blown northward and probably was, but fortunately the forms then prevalent in the South could not infect the hard spring wheats.

[4] There is a possibility that rusted barberry bushes in the New England and other Eastern States may furnish inoculum also, but this possibility seems somewhat remote.

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A NEW SCLEROSPORA FROM FIJI

WILLIAM H. WESTON, JR.[1]

As our knowledge of the genus Sclerospora increases, the species which are found infecting wild grasses in less intensively cultivated parts of the world merit special attention as possible reservoirs of infection for related crops or as possible examples of endemism. It was, therefore, with special interest that the writer learned from Mr. D. S. North, Pathologist of the Colonial Sugar Refining Company, that in the Fiji Islands a leaf-splitting disease caused by a Sclerospora occurs on the common native "reed," *Erianthus maximus* var. *Seemanni* Hack. Through the kindness of Mr. North, material collected by H. F. Clarke on the Rarawai Estate of the C. S. R. Co. in Suva, Fiji, on June 23, 1924, was forwarded for examination. As the Sclerospora proved to be a new species quite distinct from the *S. sacchari* Miyake which occurs sporadically on sugar cane in Fiji a brief discussion of its peculiarities and its relationship is presented here.

EFFECT ON THE HOST

The Erianthus plants show the effects of the fungus by becoming generally dried, withered, and brown; while the leaves, as the tissue between their bundles is disorganized through the invasion of the parasite, shred out into shrivelled brownish fibers which tangle into irregular snarls. In general, this effect agrees closely with that of the oogonial phase of most Sclerosporas on their various hosts—for example, *S. sacchari* on sugar cane, *S. miscanthi* Miyake on Miscanthus, or *S. graminicola* (Sacc.) Schr. on Setaria (Chaetochloa).

As in these other cases, also, the resting spores develop in great quantities in the frayed-out leaves of the Erianthus and can be seen with a lens as distinct resinous specks in the disorganized tissue fragments along the tangled fibers. Of the once abundant mycelium which gave rise to these spores only a few shrivelled irregular fragments now remain here and there among the host cells.

No conidial phase of the fungus has yet been encountered.

CHARACTERISTICS OF THE FUNGUS

The resting spore, as is characteristic of the genus, comprises a single oospore enclosed in a much modified, darkened and thickened oogonial wall so closely adherent that the whole forms a single resistant body.

[1] The writer acknowledges with gratitude aid from the Milton Fund of Harvard University which has facilitated this and other comparative studies of the Sclerosporas.

The shape usually is approximately polyhedral, that of a sphere with several flattened faces (Fig. 1; B 1–3, C 1–5) bordered by projecting ridges (Fig. 1; B 6–8, 11, 12, C 3, 6–8) so that in profile it appears somewhat irregularly angular with wing- or fold-like protrusions, here and there (Fig. 1; B 6–8, 11, 12, C 3, 6–8). Occasionally the shape is more irregular, elongate pyriform (Fig. 1; B 4, C 7), or unequally rounded oblong (Fig. 1; B 12, C 6, 8). The developing oogonia are modified in shape and in surface configuration in conformity to adjacent spores and to contiguous host tissue. In some cases they occur in rows or groups closely

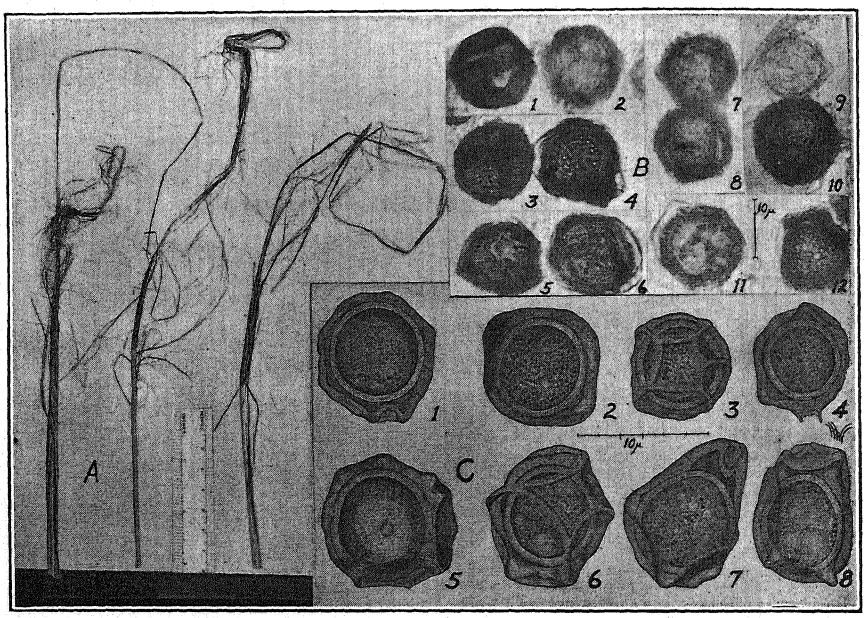


FIG. 1. A. Specimens of *Erianthus maximus* var. *Seemanni* infected by **Sclerospora northi** n. sp., from material collected by H. F. Clarke on the Rarawai Estate, Suva, Fiji, in June, 1924, showing the shredding of the leaves into snarls of vascular bundles from which the resting spores are scattered by every slight movement. Photographs, scale in inches and centimeters. B. 1–3, 7, 11. Resting spores showing the surface aspect with flattened polyhedral faces and irregular winged or ridged projections; 4–6, 8–10, 12, resting spores in optical section, showing the shape and size of the single enclosed oospore, and the characters of its wall and content; 9, a young spore in which the oogonial wall is not yet thickened, colored, and opaque, shows the interior especially clearly. Photomicrographs, scale in microns, magnification about 250 x. C. 1, 2, 4, 5. Resting spores, in optical section, showing the structure, thickness and profile of the oogonial wall, the shape and structure of the enclosed oospore and its proportions and position in relation to the enveloping wall; in 3, 6–8, combined surface and section views of cleared material show also the typical superficial configuration. Camera-lucida drawings, scale in microns, magnification about 375 x.

pressed together and with faces of contact that more or less fit each other (Fig. 1; B 7–8, C 5–6). As seen in optical section the general outline of the enveloping oogonial wall may conform approximately to that of the spherical oospore within (Fig. 1; C 1, 2, 4, 5), but the wall itself is not everywhere in intimate contact with the enclosed oospore, but arches, bulges, and folds outward into irregular, ridged prominences, leaving corresponding spaces between its inner surface and the oospore wall (Fig. 1; C 6–8). Moreover, in addition to being outfolded, the wall is rendered still more irregular through being locally decidedly thickened, at times in noticeable layers, and the surface frequently shows indefinite scabrous patches or angular or ridged protrusions.

The thickness of the oogonial wall varies, but usually is from 3 to 5 μ, less frequently in occasional thickened areas reaching 10 μ.

In color, the wall of mature spores is most commonly a dark amber, the Amber Brown, 13 k of Ridgway's "Color Standards," though ranging from the lighter Raw Sienna, 17 i, to the dark Argus Brown, 13 m. In

TABLE 1.—*Measurements of the outer dimensions of 200 resting spores of* **Sclerospora northi** *n. sp. and diameters of the enclosed oospores arranged in size classes to show frequencies*

Outer dimensions of resting spores			Diameters of oospores	
Classes	Number of resting spores in 200		Classes	Number of oospores in 200
μ	Length	Width	μ	
39 to 40.9		1		
41 to 42.9		1		
43 to 44.9	1	4		
45 to 46.7	2	9	35 to 36.9	7
47 to 48.9	4	25	37 to 38.9	16
49 to 50.9	11	23	39 to 40.9	35
51 to 52.9	2	20	41 to 42.9	43
53 to 54.9	21	46	43 to 44.9	41
55 to 56.9	19	32	45 to 46.9	30
57 to 58.9	38	20	47 to 48.9	19
59 to 60.9	28	9	49 to 50.9	8
61 to 62.9	27	7	51 to 52.9	1
63 to 64.9	17	2		
65 to 66.9	11	1		
67 to 68.9	10			
69 to 70.9	4			
71 to 72.9	1			
73 to 74.9	1			
75 to 76.9	0			
77 to 78.9	1			

darker individuals the wall can be seen through only with difficulty, even with intense illumination; in immature ones, with their pallid golden to amber color, details of the structure can be perceived with ease.

Remains of the oogonial stalk persist but rarely (Fig. 1; C 4), and only seldom are to be seen scars or impressions which might be interpreted as points where an antheridium had been attached; but, as fertilization apparently occurs very early, the thickening of the wall usually obliterates them.

The outside size of the resting spores varies greatly, ranging from as little as 40 to as much as 77 μ, with the greatest dimension, "length," falling most commonly between 57 and 60.9 μ, the dimension at right angles, "width" most frequently from 51 to 56.9 μ.

The oospore, as seen in optical section usually is central in position, of relatively large size in relation to the enclosing oogonium, and spherical in form, only rarely, in irregular resting spores being somewhat flattened or elongated, apparently restricted or constrained by the confined space in which the body has developed (Fig. 1; C 7, 8). In size the oospores are rather uniform, for, although a few may run as small as 35 μ or as large as 52 μ in diameter, the bulk of the individuals measured (*i.e.*, 154 out of 200), fall between 39 and 46.9 μ, with the size mode falling in the class (containing 84 spores) between 41 and 44.9 μ. The oospore wall is tough, dense, smooth, homogeneous or perhaps indistinctly lamellate, either hyaline or faintly tinged with pale amber color, uniformly thick, quite consistently measuring from 2 to 4.5 μ. The content of the oospore is hyaline or pallid grayish with a finely granular protoplasmic matrix in which lie denser aggregations of granules and with a clearer or vacuolate area, usually in the center, in which, in some cases, one or more refractive, perhaps golden tinged oil globules may be seen (Fig. 1; B 4, 6, C 1, 5–8).

The germination of the oospores has not been observed.

IDENTITY OF THE FUNGUS

Of the known Sclerosporas the *S. sacchari* and the *S. miscanthi* of T. Miyake in their oogonial stage show sufficient general similarity to this Fijian species to invite comparison.

S. sacchari, however, is distinguished by the fact that the resting spores are more nearly spherical, less polyhedral in shape, the oogonial wall usually much thinner, more closely conforming to the oospore within, the surface more distinctly scabrous or irregularly roughened, with ridges or winged projections, if present, usually less prominent and occupying a relatively small area, its material more transparent and of paler golden or lighter amber color. The oospores, moreover, are distinctly larger, most frequently between 49 and 54.9 μ in diameter, and with a much thicker wall, 5 to 8 μ. In like manner, though less pronounced, may be distinguished the

oogonial Sclerospora occurring on *Saccharum spontaneum* L. in the Philippines, a species very similar to *S. sacchari* but perhaps separate from it.

S. miscanthi of all the Sclerosporas, is the one the Fijian species resembles most closely, the general appearance of the resting spores, their shape, surface characters and color; and the proportion and relation of the oospore to the enveloping wall, being vary similar. Yet *S. miscanthi* seems consistently in all the material examined to have distinctly more rounded, less polyhedrally angled resting spores, and oospores which not only occupy a relatively greater space and more nearly fill the oogonial cavity but are distinguishably larger, the most of those encountered in the hundreds measured being between 47 and 48.9 μ in diameter.

With species that show such general similarity it is obviously desirable that cross-inoculation studies be made to determine whether this Fijian species may be transferred from Erianthus to sugar cane, to *Saccharum spontaneum,* to *Miscanthus japonicus* (Thunb.) Ander., and other members of the Andropogoneae, and if so whether it will retain its specific characteristics when developing on these hosts.

As far as can be determined at present, however, it seems justifiable to establish this as a distinct species. It is, therefore, described as follows:

Sclerospora northi n. sp.

Oogonium (outer portion of the resting spore) usually rounded polyhedral in shape, with several flattened faces bordered by ridges, occasionally more irregular, elongate pyriform, or unequally rounded oblong; in size ranging from 40 to 70 μ, greatest dimension, "length," most frequently between 57 and 60.9 μ, dimension at right angles, "width," most frequently 51 to 56.9 μ. Oogonial wall arched and folded out into irregular, ridged prominences leaving corresponding spaces over the oospore wall, in thickness usually 3 to 5 μ, occasionally as much as 10 μ. Wall most commonly Amber Brown, 13 k, of Ridgway, but ranging from Raw Sienna, 17 i, to Argus Brown, 13 m. Remains of oogonial stalk or of attached antheridium rare.

Oospores regularly spherical, most frequently from 39 to 46.9 μ in diameter, the mode between 41 and 44.9 μ, the extremes ranging from 35 to 52 μ; wall hyaline to pale amber, dense, smooth, homogeneous to indistinctly lamellate, 2 to 4.5 μ in thickness; content finely granular with denser aggregations and with clear usually central area with occasionally one or more oil globules.

Germination not yet observed.

Occurring on *Erianthus maximus* var. *Seemanni,* in the Fiji Islands; material collected by H. F. Clarke on the Rarawai Estate, Suva, Fiji, June 23, 1924.

Specimens deposited in the Farlow Herbarium, Harvard University.

DISCUSSION

In connection with this new Fijian Sclerospora certain points are worthy of note.

Its occurrence on an Erianthus is of interest, for the genus, on which hitherto no species of Sclerospora has ever been reported, must now be added to the long list of grasses that are known to be attacked by these mildews. It does not seem surprising, however, that this should be so, for, in the tribe Andropogoneae, the two genera, Miscanthus and Saccharum, both closely related to Erianthus, have been found to harbor two, possibly three, species of Sclerospora under natural conditions and to be susceptible to inoculation with two more.

Of the grasses of the Fiji Islands, Seemann, in his "Flora Vitiensis," of 1865, listed fifteen species, among them *Miscanthus japonicus* (*Eulalia japonica* Trin.), and sugar cane *Saccharum officinarum* L., of which he says "a purple variety attaining a height of 16 feet and of corresponding thickness is cultivated to some extent". His statement that this cane "grows, as it were, wild in various parts of the group," refers more probably to *Saccharum spontaneum* which, according to D. S. North, occurs rather commonly there. On Seemann's "*Saccharum floridum,*" No. 691, there was established by Hackel, *Erianthus maximus* var. *Seemanni,* the variety reported only from Fiji, the species itself known from Tahiti and the Society Islands as well. However, the most recent discussion of the grasses of the Fijis by Summerhays and Hubbard enumerates 59 species, but does not include the Erianthus nor any reference to Seemann's specimen on which this variety was established.

Of the Gramineae just noted, *Miscanthus japonicus* is known to harbor *Sclerospora miscanthi* in Formosa and the Philippines, sugar cane is infected by *S. sacchari* in Formosa, the Philippines, and Australia, while on *Saccharum spontaneum* the writer found a Sclerospora related to *S. sacchari* occurring commonly throughout the Philippine Islands.

In the Fiji Islands the following Sclerospora have been found by Mr. D. S. North:—The Sclerospora on the native "reed," *Erianthus maximus* var. *Seemanni,* herewith described as *S. northi;* a Sclerospora causing a leaf-splitting disease of "Veico", the wild cane (probably *Saccharum spontaneum*) which, although not yet secured for study, is perhaps the same as that which occurs in the Philippines on this host, and a Sclerospora occurring in sporadic cases on cultivated sugar cane, formerly assumed to be *S. sacchari,* but now thought by North to be distinct, as it does not cause elongation of the shoots, rarely shows the downy conidial phase, and produces oospores more readily, accompanied by more leaf splitting.

With fungi of such potentialities occurring among such closely related hosts in so small and isolated an area as the Fiji group, it seems especially

desirable that cross-inoculation experiments should be made to determine whether the species on sugar cane and *Saccharum spontaneum* are identical or are distinct; whether all these species are restricted to their respective hosts or may transfer from one to another under natural conditions or under special circumstances; and whether centers of infection on the various hosts may serve as foci from which the several fungi may pass to some cultivated crops in later agricultural developments.

As *Sclerospora northi,* so fas as known, is limited to this particular variety of Erianthus in this small group of islands, one might assume that it is endemic, but such an assumption is unjustified in the present lack of information as to the occurrence of downy mildews on *Erianthus maximus* Brongn., itself, in Tahiti and the Society Islands.

The disease caused by *S. northi,* having the same general leaf-splitting symptoms as those of the better-known malady of sugar cane, when first recognized, naturally roused the suspicion that the two might be the same and that this native "reed" growing around the plantations might serve as a reservoir of infection, greatly complicating the problem of combating cane losses. The certainty that this is a distinct species frees it from suspicion and removes it from consideration in the control of sugar-cane mildew.

As far as known, therefore, this new species is not, at present, of any economic importance as it is not seriously destructive to its wild host, nor is it passing therefrom to more valuable cultivated crops.

SUMMARY

This paper discusses a Sclerospora causing a leaf-splitting disease of *Erianthus maximus* var. *Seemanni* in the Fiji Islands.

In its resting-spore stage, the only reproductive phase yet encountered, the fungus seems distinct from *S. sacchari* and *S. miscanthi,* the two species most similar to it. It is, therefore, described as *S. northi* n. sp. and its characteristics of structure, size, and surface configuration are discussed.

The present status of this and related Sclerosporas in the Fiji Islands in regard to questions of distribution, relation to hosts, and control of disease is noted.

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PLANT-DISEASE NOTES FROM THE CENTRAL ANDES II

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In a recent paper by Chardon and Toro (*1*) an account was presented of the plant diseases encountered by Chardon during his visit to the State of Antioquia in Colombia. Thirteen diseases were reported, of which ten were hitherto not known to occur here. The present paper is based on the observations of the writer during the past year. The majority of the diseases cited are new to Colombia, while additional data are given regarding the etiology and ecological relationship of the coffee-root disease and the symptomatology of avocado leaf spot and data on other diseases.

An account of these follows:

Coffee-root disease. Studies of this disease under field conditions seem to indicate the presence of two different diseases or, at least, two different sets of symptoms of the same disease, under the vernacular name of "llaga" (canker).

The first of these is a dry rot of the upper surface roots and of the base of the trunk. No visible symptoms of the disease appear until it is well advanced, when the leaves turn yellow and fall. In some cases, a few leaves remain attached to the tree, even though they have lost their green color. If the tree is bearing fruits, these remain on the branches but do not mature normally. Examinations of diseased parts disclosed mycelial strands of a fungus resembling those described by Fawcett (*2*) associated with the black type of coffee-root disease in Porto Rico. This disease, however, is not very prevalent. It was observed only in few plantations and, generally, weakened trees are the only ones to die. Strong, well-cared-for trees do not so easily succumb to attacks of the causual organism. The writer saw trees that, according to the manager of the plantation, had been infected for two years without showing any external symptoms of the disease.

In addition to coffee, several weed and shade trees are also attacked. Strands of mycelium similar to those observed on coffee were found on the stumps of old, dead Inga trees. In one case abundant perithecia of an unidentified species of Rosellinia were found. We know of no connection between this ascigerous stage and the mycelial strands found on coffee and other plants, but other investigators assert that such connection exists.

Weather conditions seem to have very little effect on the prevalence of the disease. Since there is always considerable moisture in the soil, due to the fact that coffee is grown under shade, the disease appears in wet as well as in dry weather. Too much wet weather, however, seems to check the spread of the fungus.

The other form of "llaga" (canker) is more interesting, although rarer; while in the former case the base of the trunk turns black, with part of the

bark disintegrating, thus exposing the wood, in the latter, the bark often remains attached and acquires a greenish color. Sometimes it turns soft and gives off a pungent odor. In the majority of the specimens of this second "llaga," a Fusarium, intermixed with bacteria, was present. In some specimens, in addition to the Fusarium and bacteria, the mycelial strands of the first form were also observed. The bark commonly, though not always, turns black or the wood becomes exposed.

In a few cases perithecia of Nectria were found on the stumps of coffee roots and stems which had died from this second form of "llaga". This Nectria is identical with Chardon's specimen (Fung. of Colombia, No. 101), from Fredonia, Antioquia, which was studied at the New York Botanical Garden by the author. It agrees with the description of *Nectria coccinea* (Pers.) Fr. var. *tropica* Wr. as described by Wollenweber (*8*), from coffee material collected in Brazil. Chardon's specimen, although agreeing with the description given by Wollenweber, is very distinct from *Nectria coccinea,* as shown by the specimens, from different localities, deposited in the herbarium of the New York Botanical Garden. The "contextu radiatifibrato peritheciorum" character, so peculiar of *N. coccinea* var. *tropica,* is entirely wanting on the specimens of *N. coccinea* examined. Also the spores in the variety are smaller than those shown by the species. In view of these distinctions we propose that the variety be considered a distinct species and rename it **Nectria tropica** (Wr.) comb. nov.

It remains to be proved whether there is any connection between the Fusarium associated with the second form of "llaga" and this Nectria, and whether this Fusarium is a primary pathogene or only invades wounds received by the trees during cultural operations. An investigation of these relationships has been initiated.

Cercospora spot of coffee. This disease was observed for the first time this year. In a great majority of the grains classed as second-grade, due to adherence of the parchment, lesions and spores of the Cercospora type were found. In the berries the fungus forms black blotches which may cover as much as half of the surface. These blotches occur more frequently on the upper side where the sun causes early ripening. In the leaves the spots are circular, at first brown, later becoming almost white in the center with a definite brown margin. When dry, the central part does not break away as happens in the case of the leaf spot caused by the fungus *Omphalia flavida* Maubl. & Rangel. The cause of this tronble is a fungus first described from Jamaica under the name of *Cercospora coffeicola* Berk. & Cke.

Field observations have shown that the infective capacity of the causal organism depends on conditions affecting the trees. The disease is more prevalent in places where the shade is insufficient, where cultivation has

been neglected, and where, in general, a weakened condition of the tree prevails.

Top rot of sugar cane. A serious outbreak of this disease was reported during the month of October on a farm at Bello, near Medellin. Other sporadic cases have been reported from Sonsón and other places. An examination of the field in Bello showed a loss of at least 60 per cent of the crop. At that time the weather was very rainy and the soil was full of stagnant water. At first, therefore, the trouble was thought due to excessive soil water. A closer examination of the stools showed that the plants were suffering from a disease similar to that described by Matz (*3*) as the dry top rot of sugar cane in Porto Rico. There was a wilting and drying of the central leaves, a stunting of the cane stalks, and a dry rot of the uppermost joints. The length of the internodes of some of the stalks was so reduced that there was an unusual number of nodes, while root development was greatly increased. Microscopical examination of the stalks showed the vascular bundles all clogged with orange red, spherical masses. This condition was observed in plants growing on the hilly parts of the plantation. Those growing in the valley showed the characteristic yellowing resulting from excessive moisture.

Since top rot may be caused by several organisms, the writer is not certain that the Antioquian disease is the same as that described by Matz from Porto Rico. The fact that a heavy infestation of borers is always present in all cane plantations in Antioquia and that several different fungi may cause the spherical, orange-red masses in the vascular bundles and other tissues of the cane stalk, makes it necessary that a more careful study of the disease be made before giving any definite conclusions as to its exact causation.

It is impossible to consider at the present time the matter of varietal resistance to the disease, since the four varieties planted here are the most susceptible to Matz's disease. These are the Otaheiti, Rayada, Christalina, and Cavangerie, the last one being the most resistant. Since cane production in Antioquia is dependent on these varieties—for lack of others to take their places—if the disease continues to check production and dissemination, it will be a matter of years before other cane varieties can be established and methods of control be developed.

Red rot of sugar cane. Several complaints came to the writer that the yields of "panela" (loaf-sugar) were considerably decreasing on account of a stunting and drying of the canes and the consequent small yields. Visits to some of the plantations showed that this disease was due to a number of causes, chief among which were defective cultural methods, enabling the facultative parasites to attack the already weakened plants. Examination of the roots of several of these sick plants showed a reddish

coloration accompanied by death of the great majority of them. A microscopic examination showed the presence of macroconidia of a *Thielaviopsis*, very probably *Th. paradoxa* (Des.) v. Hohn., the cause of the pineapple disease of sugar cane in other cane-producing countries.

This fungus is primarily a cutting-inhabiting organism and is the cause of much poor germination in cane fields. However, several cases are known in which the fungus has attacked standing canes, especially in wet weather. In cutting, the fungus enters through the wounds received during planting operations, but in standing cane it is difficult to ascertain how the organism enters the plant, unless it be through the borer holes or through wounds received by the plant from the attacks of other organisms or during cultural operations. In view of our knowledge of cane diseases in Antioquia, we are not in a position to express definite conclusions as to the exact cause of this red rot. The fact that root troubles in sugar cane may result from a number of causes and that, even though fungi are found associated with this trouble, which, under proper management of the plantation, are unable to cause any great losses, makes it the more difficult to ascertain the exact nature of the diseased conditions.

Avocado tar spot. This is a leaf spot caused by the fungus *Phyllachora gratissima* Rehm. In Antioquia it is the worst malady of the avocado, *Persea persea* (L.) Cock. It causes considerable falling of the leaves, in some cases killing the trees because of their inability to produce new folliage.

The fungus first makes its appearance on the young leaves at any time before they reach maturity, in many cases killing the young leaves before these have attained their full size. This results in reduced vigor and subsequent attack at the tops by secondary fungi. Black, conspicuous, shining spots appear on the upper side of the leaves with corresponding yellowish spots on the under side. The stromata are arranged in circles, sometimes separated by brownish zones, and so abundant as to cause the leaves to fall prematurely. In most cases the leaves turn brown before falling.

Leaf spot of cauliflower. During a visit to the Granizales section, the place where most of the vegetables consumed in Medellin are produced, several vegetable diseases were encountered. The most conspicuous of these was the black leaf spot of cauliflowers. The whole head was in many instances covered with a brownish black crust formed by the conidiophores and spores of the fungus *Alternaria brassicae* (Berk.) Sacc. Weather conditions seemed to favor the development of this organism, since plants which were exposed to the sun at all times, and consequently drier, were less affected by the disease.

Ring spot of cabbage. Concentric, circular, light brown spots, surrounded by greenish borders were observed on cabbage leaves. Closer examination of the spots showed them to be closely studded with perithecia of the fungus *Myosphaerella brassicicola* (Duby) Lindau.

Cercospora leaf spot of lettuce. A severe attack of leaf spot on lettuce was encountered. The organism found associated with it agrees with *Cercospora lactucae* Stevenson described from similar material in Porto Rico (*5*). Welles (*7*) also described another Cercospora on lettuce and named it *C. lactucae* Welles. Both of the descriptions agree with that of *C. longispora* Cug. as given by Traverso (*6*). The name *C. longispora* was previously occupied and Saccardo (*4*, p. 607) changed the name to *C. longissima* (Cug.) Sacc. P. Hennings described another Cercospora on lettuce, which he named *C. lactucae* P. Henn. before Stevenson and Welles described their species. *C. lactucae* P. Henn. is very distinct from *C. longissima*. Since *C. longissima* is an older name, the fungus from Porto Rico, Philippines, and Colombia should be referred to this name.

Leaf mold of tomato. Garden tomatoes were found heavily infested with the fungus *Cladosporium fulvum* Cke. The disease is more prevalent during rainy weather.

Bean anthracnose. All varieties of beans are attacked by the anthracnose fungus *Colletotrichum lindemuthianum* (Sacc. & Magn.) Brio. & Cav. The lesions on the pods differ according to the variety attacked and the prevailing climatic conditions.

Wither tip of mango. An investigation of the premature falling of flowers and of young fruits of mango (*Mangifera indica* L.) showed that a fungus *Colletotrichum gloeosporioides* Penz. was always associated with the trouble. Besides producing these symptoms, the fungus also causes a wither tip of the young branches, so that considerable damage is done to the tree accompanied by the very scarce production of fruits.

Bunchy top of carnation. Associated with this trouble was found the fungus *Heterosporium echinulatum* (Berk.) Cke. Young leaves were covered with a blackish crust formed by the conidiophores and spores of the fungus, while older leaves were dead and the plants acquired a broom-like appearance, due to abnormal growth of lateral branchlets.

Banana anthracnose. Heavy infections of fruit rots were observed on ripe market bananas. Abundant acervuli and spores of the fungus *Gloesporium musarum* Cke. & Massee were found on the lesions. It seems that this fungus is capable of causing the trouble on ripe fruits that have been injured.

Minor diseases. Among the other diseases occasionally observed were: blue mold of oranges caused by *Penicillium digitatum* (Pers.) Sacc.; leaf spot of pepper caused by *Cercospora capsici* H. & W.; flower-head blotch of the grass *Sporobolus berteroanus* (Trin.) H. & C. caused by *Helminthosporium ravenelli* Curt.; black leaf spot of *Carica papaya* L. caused by *Asperisporium caricae* (Speg.) Maub. This fungus has been reported under a variety of names such as *Cercospora caricae* Speg. and *Puccinio*p-

sis caricae Earle. A heavy infection of Inga leaves by the alga *Cephaleurus virescens* Kunze was observed during dry weather.

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ATTENUATION OF CURLY-TOP VIRUS BY RESISTANT SUGAR BEETS WHICH ARE SYMPTOMLESS CARRIERS

C. F. LACKEY[1]

INTRODUCTION

Since the previous work was reported on attenuation of curly-top virus by resistant sugar beets showing mild to moderately severe symptoms (3), other beets have been found in the field which attenuate the virus without showing any visible manifestation of its presence.

The first work on the attenuation of the virus of sugar-beet curly top was reported by Carsner and Stahl (4). They found *Chenopodium murale* L. to have a marked effect on the virus. Carsner (2) found that *Rumex crispus* L. and *Suaeda moquini* Greene also attenuated the virus when it was passed through them. Carsner and Stahl (4) failed to detect any well-defined symptoms of curly top on *C. murale*. In the writer's work, these observations are confirmed; numerous experiments indicate that under certain conditions *C. murale* not only attenuates the curly-top virus, but also acts as a symptomless carrier.

Several examples have been reported in which plants of one genus act as symptomless carriers of a virus affecting plants of other genera. Johnson (6) reports apparently healthy potatoes harboring a virus which causes a disease of tobacco. Elmer (5) and Nishimura (8) record cases in which *Physalis alkekengi* L. also harbored a virus of tobacco without apparent symptoms. Atanasoff (1) found that some varieties of potatoes may carry the virus of stipple streak without any visible effect, but that when the virus is transferred to potatoes of a different variety conspicuous symptoms may become visible. Except for the plants reported by Carsner and Stahl (4) and Carsner (2), no attenuation of the viruses harbored by these various hosts has been recorded.

While continuing some investigations on the attenuation of the curly-top virus by resistant sugar beets (3), some individual plants were discovered in the field, among the most resistant strains of sugar beets, which were apparently healthy, having no visible curly-top symptoms whatever. Each beet in the field had been inoculated by caging two viruliferous leaf hoppers (*Euttetix tenellus* Baker) on it for 24 hours while it was very young, and the plants had also been exposed to a fairly heavy natural infestation of leaf-hoppers. These inoculations might be expected to produce severe curly-top infection in plants of ordinary susceptibility and thus cause the apparently healthy individuals to attract attention. In this same

[1] The investigations herein reported were conducted under the supervision of Dr. Eubanks Carsner.

field, in all the other strains which are susceptible, or at least less resistant than the strains in which the seemingly healthy individuals occurred, all plants exhibited symptoms of curly top. It is evident from these facts that the symptomless plants had been well inoculated.

The facts discovered by other workers, as previously noted, regarding the harboring of viruses without apparent evidence of their presence (1, 6, 7, 8) suggested that a similar situation might exist in the case of the apparently healthy resistant beets. The fact also was kept in mind that they might have resisted infection entirely. Earlier research (3) indicated that when resistant beets act as carriers of the curly-top virus they may produce an attenuating effect. The severity of the symptoms on the plants inoculated corresponds rather closely to the degree of severity exhibited by the resistant beets from which the virus was taken. The possibility was therefore suggested that in case the virus was harbored by the symptom-free resistant beets here under consideration it might be extremely attenuated.

EXPERIMENTAL TESTS

Experiments were conducted to determine whether or not the virus was present in these apparently healthy sugar beets and, if present, the degree to which it was attenuated. Some of the youngest inner leaves were taken from these symptom-free sugar beets, and nonviruliferous leaf hoppers were caged on these detached leaves for 2 to 3 days. These insects were then caged in lots of 10 on healthy, young, four-leaf susceptible beets. For controls, similar plants were inoculated with leaf hoppers which had fed on severely diseased leaves of a susceptible beet. All inoculated plants were kept under conditions favorable for the development of curly top. In 10 to 37 days all the plants inoculated with virus from these symptomless carriers became diseased, but they were not so severely affected as the controls. The average incubation period of the control plants was shorter than that of the others, all controls showing the first symptoms in less than 10 days. There was a noticeable variation in the degree of attenuation among the different individuals in these strains of symptom-free beets. For example, in one case the virus produced moderately severe symptoms in 10 days on susceptible beets, although these symptoms were not so severe as those on the controls. In contrast to this the virus from another strain of resistant carriers produced mild and very inconspicuous symptoms of curly top in 37 days. Furthermore, about five weeks later, the faint symptoms on the mildly affected beet had disappeared entirely. It apparently had completely recovered. Repeated tests yielded similar results. However, in most cases the virus from the symptom-free beets produced symptoms almost as severe as the virus from the beets showing mild to moderately severe effects (3).

While, in breeding sugar beets for resistance to the curly-top virus, the appearance of symptomless carriers has never been such a confusing factor as has been the case with this phenomenon in certain other virus diseases, it seems quite possible that one not familiar with the fact of their occurrence might be led to consider such apparently healthy beets to be immune from the disease.

Aside from any practical application which the results of these experiments may have, they demonstrate the interesting fact that under certain conditions some resistant sugar beets acting as symptomless carriers may harbor the curly-top virus in a state almost as virulent as do those resistant beets which exhibit symptoms ranging from mild to moderately severe.

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THE HISTORY AND DISTRIBUTION OF *PHOMOPSIS PSEUDOTSUGAE* IN EUROPE[1]

MALCOLM WILSON AND GLENN GARDNER HAHN

INTRODUCTION

A very important disease, primarily of the Douglas fir, *Pseudotsuga taxifolia* (Lam.) Britt.,[2] a conifer which, since the World War, has been extensively planted in Great Britain, is a canker (Fig. 1) and die-back attributed to *Phomopsis pseudotsugae* Wilson (42). The canker is essentially a disease of young trees. The die-back of the tops and branches, which also occurs on larger and mature individuals, may become particularly serious in young plantations of the green form (*Pseudotsuga douglasii* Carr.).[2] The latter type of disease, when occurring in connection with stem canker on trees approximately less than eight feet in height, may result not only in a much reduced final stocking, so far as the mature stand is considered, but economic loss, as well, by the death of trees which otherwise might have been of value as thinnings.

As yet, the Douglas fir canker fungus, described and figured by Wilson (40, 41, 42) as the Phomopsis disease of Douglas fir and other conifers, is not known in North America. In the light of our experience of the highly dangerous nature of imported parasites as the result of their introduction into a new environment with a consequent change in their virulence and life habits, we are forced to recognize in the Douglas fir canker of Great Britain a potential danger to the Douglas fir in North America. Considering the value of this tree in the United States from the standpoint of merchant-

[1] Paper by Dr. Malcolm Wilson, Mycological Department, University of Edinburgh, at the Royal Botanic Garden, Edinburgh, and Glenn Gardner Hahn, Office of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

[2] In the United States only one species of Douglas fir, *Pseudotsuga taxifolia*, is recognized. In Europe this American species is considered to include two distinct species —*P. douglasii*, (commonly called coast, Pacific coast, Oregon, or green Douglas fir), and *P. glauca* Mayr. (commonly called mountain, Rocky Mountain, Colorado, or blue Douglas fir). *P. douglasii* var. *caesia* Schwer. is regarded as the Fraser River form. This name has been pointed out by Boyce (3) as a misnomer. Biological differences tend to support the European species concept of Douglas fir. These differences have been indicated by Boyce (3) as the result of his observations of the diseases of Douglas fir in Great Britain and Denmark. For convenience, in this paper, the European usage is followed.

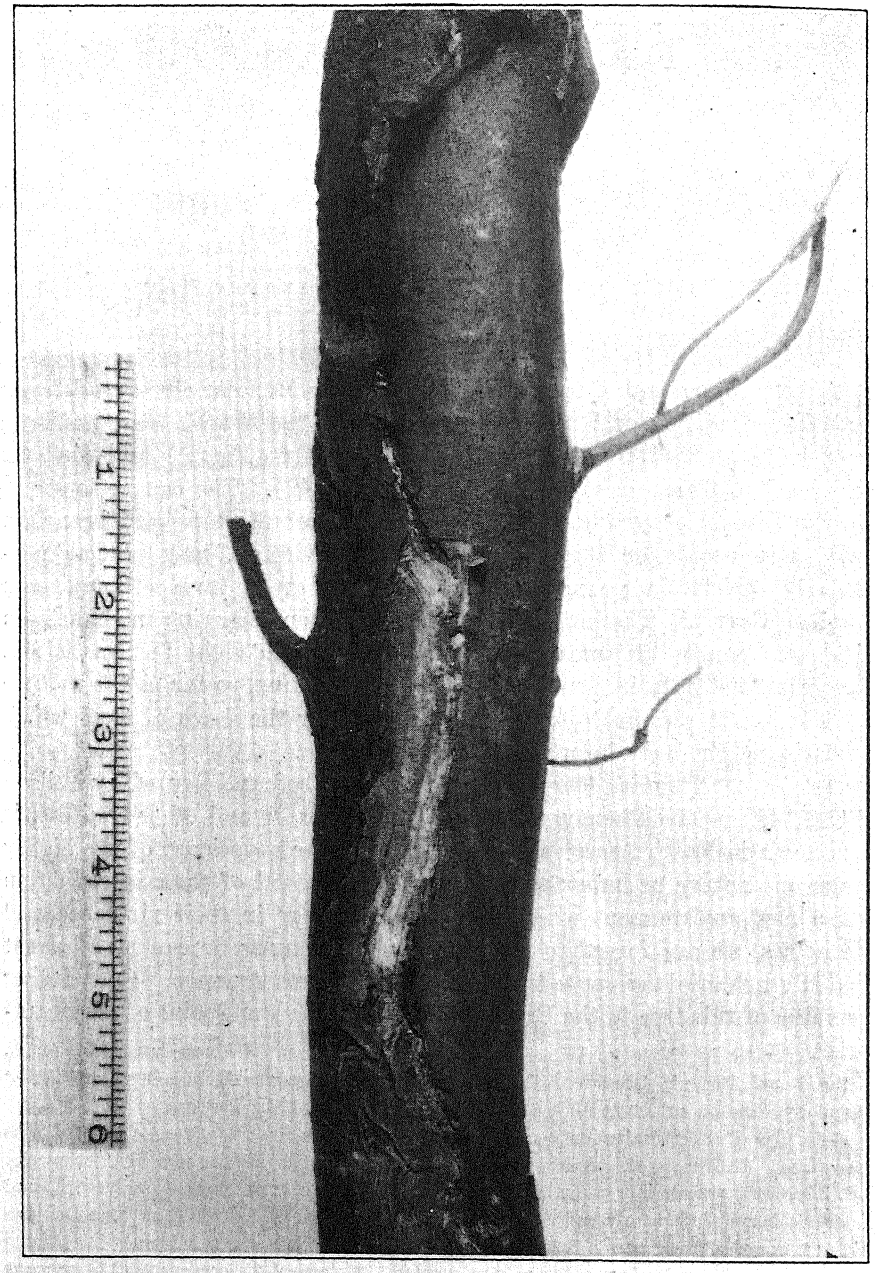


FIG. 1. Typical canker with the fruiting bodies of *Phomopsis pseudotsugae* on the green form of Douglas fir (*Pseudotsuga douglassi*) collected by Perley Spaulding at Prestwick, Ayrshire, Scotland. May, 1923. Approx. $\frac{2}{3}$ nat. size.

able timber and as a forest crop of the future, any disease, which may be looked upon as a menace to the species, should be regarded with close scrutiny and suspicion.

On account of the great importance of the Douglas fir canker, the junior author has been working in cooperation with Dr. Malcolm Wilson in further investigating the disease to prove that it is actually caused by *Phomopsis pseudotsugae,* and to discover the method of infection, the origin of the disease, its original host, its present distribution, and its relation to somewhat similar diseases which have already figured in European mycological and forestry literature. The last three subjects will be discussed in this paper.

IMPORTANT EUROPEAN DISEASES OF CONIFERS RESEMBLING THE DOUGLAS FIR CANKER

Wilson (42) and Boyce (3) have already discussed the close relationships and the large amount of confusion which has accumulated in the literature for approximately the last half century, concerning the identity of *Phoma pitya* Sacc. (32, p. 126) and *P. abietina* Hartig (11, p. 124–126), both attributed parasites of the Douglas fir and other conifers. Since the fairly recent description of *Phomopsis pseudotsugae* by Wilson in 1920 (41, 42), this fungus has been regarded by some investigators as synonymous with the older fungi originally described by Saccardo and Hartig. It therefore became highly necessary from a practical standpoint to differentiate the newly described organisms from the older and well-known parasites which may have been introduced into the United States in previous years on ornamental or plantation stock of various conifers.

In 1885, Rostrup reported *Phoma pitya* as a parasite of plantation stock of Norway spruce (29, p. 10) and later recorded it on other conifers in Denmark (30, 31). Since this time confusion has been very evident. The Danish mycologist regarded his organisms as synonymous with the classical fungus *P. abietina* which is attributed as the cause of the "Einschnürungskrankheit" of the silver fir. Following Rostrup, diseases of the Douglas fir generally described as a girdling or die-back of the shoots were reported by Somerville (34) in Scotland (1898), by Lindau (20) in Germany (1908), by Schwappach (33) in North Germany (1909), by Visart and Bommer (39) in Belgium (1908), by Farquharson (7) in Scotland (1911), by De Koning (18, p. 160) in Holland (1922), by Neger (25, p. 173) in Germany (1924), and by Fabricius (6) in Denmark (1926). These authors all identified the organism associated with the diseases as *P. pitya,* a fungus on Scots pine, *Pinus sylvestris* L., which had been briefly described by Saccardo.

Rostrup and Schwappach regarded *Phoma pitya* as identical with Hartig's fungus, *Phoma abietina,* on the silver fir. Other European investigators, however, regarded the latter fungus as a distinct species. It was reported by Böhm (2) in 1896 and Hausrath (12) in 1923 on the Douglas fir in Germany. In France (1890) Hartig's fungus was regarded as *Fusicoccum abietinum* (Hart.) Prill. et Del. by Prillieux and Delacroix (28), and Maublanc (22). Mer (23, 24) published extensively on the disease with regard to its pathological symptoms and histology and made, incidentally, a brief reference to his inoculation experiments. This investigator, together with Henry (13), seemed to prefer to call the fungus by its original name *Phoma abietina.* In recent years *P. abietina* has been reported by Doidge (5) from South Africa (1924) and Stevenson (35, p. 3) from the United States (1926). Considerable uncertainty, however, appears to have existed in the minds of European mycologists over the exact identity of *P. pitya* and *P. abietina.* This uncertainty was expressed in 1897 by Tubeuf and Smith (38, p. 465). Tubeuf (37), in his text book, had drawn attention to Rostrup's description of *P. pitya* quoted above and mentioned that it closely resembled *P. abietina.* Smith, in a footnote in the English translation, suggested that there had been some confusion between the two species. This confusion, no doubt, was largely due to the scant description of the former fungus. The organism recognized in different parts of Europe as *P. pitya* and regarded as being identical with Saccardo's original fungus collected by Magnus apparently held certain marked morphological resemblances to Hartig's fungus. Further, the pathological host symptoms of Hartig's fungus appeared to be identical with those attributed to the older fungus *P. pitya.* Although Hartig described this fungus only on *Abies pectinata* DC., it was also regarded by investigators as occurring on other conifers, including Douglas fir. In 1913 Lind (19), in going over Rostrup's herbarium, made the new combination of *Phomopsis pitya* (Sacc.) Lind based on the collections of *P. pitya* identified by Rostrup; he regarded Rostrup's fungus as identical with the *P. abietina* of Hartig.

In 1918 Grove (8), in discussing the identity of *P. pitya,* stated that Saccardo's fungus was undoubtedly *Sclerophoma pitya* (Sacc.) Died. and that possibly "*Phomopsis pitya,* Lind = *Fusicoccum abietinum* Prill. et Delacr." might be synonymous. Three years later (9) he made the combination *Phomopsis abietina* (Hart.) Grove, including *Phoma abietina* Hart. and *Phomopsis pitya* Lind as synonyms. His description of the fungus, on which this combination was based, was apparently not derived from a study of authentic material on the silver fir from either Germany or France, where Hartig, and Prillieux and Delacroix, respectively, had described the organism, but from diseased specimens of Douglas fir from

Scotland. His description of this Douglas fir Phomopsis differs considerably from that given by the German and French authors of the continental fungus on *Abies pectinata.*

Hickel (14), in his monograph of the Douglas fir (1922), mentions the diseases of that species in Europe. He lists *Phoma pitya, P. abietina,* and *Phomopsis pseudotsugae* as synonymous. Jørstad (17, p. 169–170) in 1925 reports that while he has not seen Norwegian material of *P. abietina* on Abies, the *P. pitya* destroying the tops of silver firs at Sand in Ryfylke, recorded by Schøyen, may be *Phomopsis abietina* (Hart.) Grove. He further states, "*Phomopsis pseudotsugae,* which is known as producing similar girdling disease primarily on *Pseudotsuga douglasii,*" has not been recorded from Norway, although a twig disease resembling it has been observed.

A perusal of the foregoing discussion clearly demonstrates the confusion in the literature relative to the exact identity of the three organisms to which similar girdling and die-back diseases of the Douglas fir and other conifers have been attributed. The solving of this problem was of particular importance to North America because of the acute necessity for complete recognition of the European parasite *Phomopsis pseudotsugae* on conifers in the United States and Canada.

THE IDENTITY OF PHOMA PITYA

For a full discussion of the identity of *Phoma pitya* and *P. abietina* and their relationships to *Phomopsis pseudotsugae* the reader is referred to a recent paper published by the authors (44). As a result of the examination of the type specimen of *P. pitya,* the fungus was found to be neither a Phoma nor a Phomopsis but a Sclerophoma. Accordingly, the fungus has been described as *Scl. magnusiana* Wilson et Hahn in honor of Professor Magnus, its discoverer in 1878. The following synonyms are given: *Phoma pitya* Sacc., *Scl. pitya* (Sacc.) Died., non *Scl. pitya* (Thüm.) v. Höhn.

Sclerophoma magnusiana produces its spores, so far as could be ascertained, by a budding process from the hymenial cells lining the spore cavity. No evidence of endogenous spore origin as reported by von Höhnel (15) for this genus could be observed. Van Luyk (21) describes a similar budding process for spore formation in *Scl. pityophila* (Cda.) v. Höhn., and Archer (1, p. 11) reports the same for *Scl. concaviuscula* (E. et E.) v. Höhn, *Dothichiza minor* (E. et E.) v. Höhn, and *Scarcophoma miribelii* (Fr.) v. Höhn. Archer has stated that it is to be regretted that certain genera drawn from the genus Phoma by von Höhnel and placed in the Endogenosporae can not be retained in their original form as erected by him. A further critical morphological study, together with a cultural

study of the various species of Sclerophoma (with special reference to those occurring on confers), is necessary to supplement observations already made by von Höhnel (15, 16), van Luyk (21), Petrak (26, 27), and Archer (1). The validity of the genus Sclerophoma is still somewhat uncertain and until further investigation has been undertaken the assignment of *Scl. magnusiana* to it must be regarded as provisional.

Sclerophoma magnusiana is known to occur on Scots pine as a saprophyte. While certain of the other species of Sclerophoma reported on conifers may be suspected of being weakly parasitic, there have been no observations made which would suggest that *Scl. magnusiana* is anything but a saprophyte.

THE IDENTITY OF ROSTRUP'S SPECIMENS OF PHOMA PITYA

The fungi which Rostrup recognized as parasites on the terminal shoots and branches of the Douglas fir and other conifers and which he identified as "*Phoma pitya* Sacc. = *P. abietina* Hartig," have been examined and the details of this examination have been recently published (10) by the junior author. It was found that Rostrup had included a number of fungi under this identification. These fungi included *Phomopsis strobi* Syd., *P. pseudotsugae,* (?) *P. juniperovora* Hahn, a *Phomopsis* sp. and *Sclerophoma* spp., including very probably *Scl. pityophila* (Cda.) v. Höhn, all of which Lind (19) called *Phomopsis pitya* (Sacc.) Lind. The *Phomopsis* sp. included in the above collections has been identified with a fungus originally described on cones as *P. conorum* (Sacc.) Died. (*Phomopsis pitya* Lind, pro parte). The emended description of the fungus is based on freshly collected Scottish material occurring on the Douglas fir, which is morphologically identical with the Danish specimens collected by Rostrup. *P. conorum* is regarded as a saprophyte, probably becoming only weakly parasitic under certain host conditions. Hahn (10) frequently observed the organism in connection with frost injury amongst nursery and plantation stock of Douglas fir associated with the killing back of the terminal shoots, which closely resembled the die-back on the same host caused by *P. pseudotsugae* (Fig. 2, C). *P. conorum* has never been observed associated with body cankers (Fig. 2, D) on the trunks and larger branches of the Douglas fir.

THE IDENTITY OF PHOMA ABIETINA

The actual type specimens of this fungus, identified by Hartig as *Phoma abietina* and by Prillieux and Delacroix as *Fusicoccum abietinum* (Hart.) Prill. et Del., were not examined, but authentic material actually collected and identified by the German author and by Magnus, and field material from the Jura (Fig. 2, A) freshly collected and received through the cour-

tesy of Professor Guimer, of the College of Forestry, Nancy (1926), were available for critical study.

The above-mentioned material answered exactly the description of the fungus given by the German (11) and French authors (28). The writers are, however, inclined to regard the fungus as a Phomopsis and have described it as such (44), giving it the new combination *Phomopsis abietina* (Hart.) Wilson et Hahn with the following synonyms: *Phoma abietina* Hart.; *Dothiorella pitya* Prill. et Del., nec Sacc.; *Fusicoccum abietinum* (Hart.) Prill. et Del.; nec *Phomopsis abietina* (Hart.) Grove. As has been pointed out above, Grove (9) in making his new combination, based his description not upon authentic material collected on the silver fir in France or Germany, but on specimens collected on the Douglas fir in Scotland. The morphological characters given by him in the description showed a marked difference from those given by Hartig and the French authors in their original descriptions.

The only typical material of *Phomopsis abietina* seen by the authors has been collected in Germany and France. The fungus has not been observed so far on Abies or on Douglas fir in Great Britain, although the organism may possibly be present in that country. The authors would be somewhat inclined to regard the fungus as a continental species, not coming normally so far north as the British Isles. Jørstad (17, p. 169) reports that he has never observed the organism in Norway. Professor Ferdinandsen and Magister Rostrup of the Plantepatologisk Laboratorium at Copenhagen, in correspondence with the junior author, state that they have not observed the typical symptomatology of *P. abietina* on the silver fir in Denmark.

Phomopsis abietina is the attributed cause of the classical "Einschnürungskrankheit" (Fig. 2, A) figured and described in detail by Hartig (11) and Mer (23, 24). Considerable controversy has occurred in France concerning the seriousness of the disease. In certain years the fungus seems to have strongly manifested itself in France and in Germany associated with a girdling of the smaller branches and stems of the silver fir. Large diameters were not attacked and trees were never killed outright by the disease. Today the disease on the Continent, while recognizable, is not regarded as a particularly serious one.

Whether *Phomopsis abietina* occurs on the Douglas fir on the Continent is problematical. Böhm (2), Schwappach (33), and Hausrath (12) have all reported it on this host in Germany. It is very possible that they might have confused other Phomopsis species, e.g., *P. pseudotsugae* or *P. conorum,* with this fungus. As Boyce (3, p. 4) has pointed out, Böhm's (2) description of the effect of the fungus on the host strongly suggests *P. pseudotsugae.*

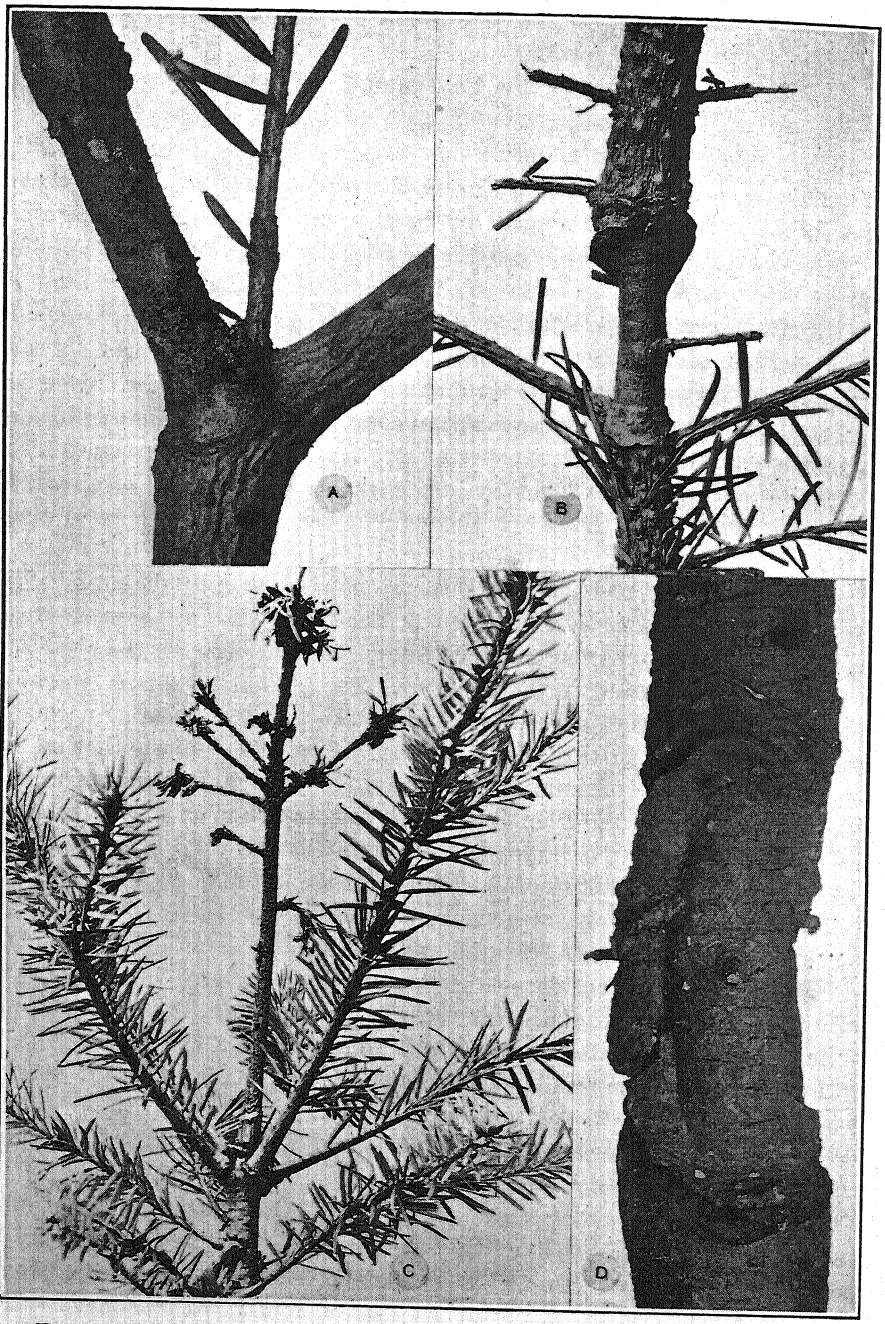


FIG. 2. A. Girdling or "Einschnürungskrankheit" of a smaller branch of silver fir (*Abies pectinata*) attributed to *Phomopsis abietina*. Freshly collected material from the Jura, France. Natural size. B. Girdling of a four-year-old tree of Douglas fir caused by *Phomopsis pseudotsugae*. Natural size. C. Die-back of the leading shoot of a four-year-old tree caused by *Phomopsis pseudotsugae*. Practically the entire terminal growth is dead.. ½ natural size. D. The canker has killed an eight-year-old plantation tree of Douglas fir by practically girdling the trunk.

Specimens reported by Doidge (5) of *Phomopsis abietina* from South Africa have not been examined. The specimen of this fungus cited by Stevenson (35) in the United States on *Abies veitchii* has been examined through the courtesy of Dr. H. C. Young, Ohio Agricultural Experiment Station, and was found not to be *P. abietina.* The identity of this fungus will be discussed in a later paper.[3]

PHOMOPSIS PSEUDOTSUGAE, A PARASITE OF THE DOUGLAS FIR

A consideration of the foregoing, together with the descriptions of the organisms, *Sclerophoma magnusiana* (*Phoma pitya*), *Phomopsis abietina* (*Phoma abietina*), and *Phomopsis pseudotsugae,* recently published (44), indicates the separate identity of the three coniferous fungi.

Sclerophoma magnusiana, so far as observations have been made, is not associated with a girdling of the stems of Scots pine. The *Phoma pitya* reported by Neger (25, p. 173) on Douglas fir and *Pinus strobus* as causing an *Einschnürungskrankheit* may have been a Phomopsis, possibly *P. pseudotsugae.* Sydow (36) has described *P. strobi* on white (Weymouth) pine from North America but he does not mention an attendant girdled condition of the host.

Phomopsis abietina, on the other hand, is a species associated with a typical *Einschnürungskrankheit* (Fig. 2, A) but the organism has never been actually shown to be a definite parasite. The fungus has not been observed occurring on cankers on the larger diameters and trunks. Only the smaller twigs and branches are girdled and, as a result, the parts beyond are slowly killed, becoming reddish brown and showing strikingly amongst the healthy green foliage. Cultural studies of this fungus from authentic material have been made and these have shown distinct differences from *P. pseudotsugae.* These cultural distinctions, in addition to differences in morphology, entirely dissipate all former confusion attendant on the identity of the two Phomopsis species.

Phomopsis pseudotsugae may be regarded as a true parasite. Experiments[4] performed under natural conditions have given positive results. The Douglas fir parasite causes three types of lesions,—first, a definite die-back of the young branches and shoots (Fig. 2, C); second, a typical girdling (Fig. 2, B) wherein the death of the tissues beyond the girdled area takes place much more slowly than the tissues involved in the preceding die-back; and third, a definite canker (Fig. 1; Fig. 2, D) of limited growth

[3] Hahn, G. G. Life-history studies of the species of Phomopsis occurring on conifers. (Unpublished manuscript.)

[4] The results of these experiments conducted by the junior author in cooperation with Dr. Wilson will be published shortly. (Parasitism of *Phomopsis pseudotsugae,* unpublished manuscript.)

on the larger branches and trunks, which normally develops during the winter and spring following infection and stops completely when the host resumes active growth in late spring. Boyce has pointed out (3, p. 6) that the fungus *Neofabraea malicorticis* (Cordley) Jackson, causing a biennial canker on apple trees in the Pacific Northwest, behaves in much the same way. Wilson (42) has published with considerable detail descriptions of the lesions caused by *P. pseudotsugae.*

With regard to the question concerning the origin of the Phomopsis disease of conifers, the authors are of the opinion that very probably *Phomopsis pseudotsugae* is a continental fungus, present in Europe in former years and occurring on a number of coniferous hosts either saprophytically or parasitically. It was collected by Rostrup in 1892 on *Abies pectinata* and has been recorded on this host and on European larch by Wilson (42); it also has been recently found as a parasite on *Cedrus atlantica* Manetti (1926), on *Larix sibirica* Ledeb. (1927) and on *Cedrus deodara* Loud. (1928). These trees are Old World species and it is very possible that one or more of these trees were the original hosts of the fungus. More than likely the organism was recognized in previous years but has been hidden under other identifications, e.g., *Phoma Pitya and P. abietona.*

Up to the present time the fungus is known to occur on the following hosts: the green form of Douglas fir, *Pseudotsuga douglasii,* the blue form *P. glauca,* the variety *caesia* of *P. douglasii, Larix leptolepsis* Murr. (Japanese larch), *L. europaea* DC. (European larch), *L. sibirica* (Siberian larch), *Abies pectinata* (silver fir), *Cedrus atlantica, C. deodara* and *Sequoia gigantea* Torr.[5] On *Cedrus atlantica* the fungus caused a definite girdling and a die-back of the terminal shoots. Among the hosts listed the greatest amount of damage occurs on the green Douglas fir. In Britain the blue form apparently is only occasionally affected. On the other hand, the latter host and particularly the intermediate forms of Douglas fir are repeatedly and seriously attacked by the American needle cast parasite, *Rhabdocline pseudotsugae* Syd., which, until 1927 (43), was confined to a single localized infection area in southern Scotland, but by that year was reported from several different sources in England (4). *R. pseudotsugae* is a very interesting case of an imported parasite which in its new environment generally attacks only one of the host forms which it ordinarily parasitizes in North America. Fortunately the more valuable green Douglas fir (Pacific coast form) has remained practically unaffected in Great Britain. The needle-cast disease is not known in Ireland or on the Continent.

[5] Specimens collected by Dr. E. P. Meinecke, Benmore, Argyllshire, Scotland, September, 1928. Culturally this form of *Phomopsis pseudotsugae* on Sequoia was identical with forms isolated from Douglas fir, larch, and cedar. Artificial inoculations made by Hahn with the *Cedrus deodara* form on nine-year-old potted trees of *C. atlantica* have given positive results.

In Norway, Hahn (1927) observed the blue form of Douglas fir vigorously attacked by *Phomopsis pseudotsugae,* which caused a serious die-back of the terminal shoots and branchlets extending throughout the tree. This injury was not accompanied by a typical trunk canker such as occurs on the green form of Douglas fir in Great Britain. A trunk and branch canker of common occurrence was observed however by him on the blue form associated with *Dasyscypha resinaria* (Cke. et Phill.) Rehm, and a *Dasyscypha sp.* (? *D. subtilissima* (Cke.) Sacc.). The parasitism of these Discomycetes has been briefly alluded to by Jørstad (17, p. 158–9) in his discussion of pine cankers. Further investigation of these attributed parasites appears to be necessary.

In consideration of the relationship between the parasite *Phomopsis pseudotsugae* and Douglas fir, the instance may very probably be considered one wherein an introduced host becomes subject to the parasitic attack of an organism already present in the country. Lesions of young and large trunks of the Douglas fir similar to those produced by *Phomopsis pseudotsugae* have not been observed hitherto in North America nor is there any record of the fungus occurring there. It appears unlikely that the fungus is an imported species introduced into Europe on Douglas fir from America.

Phomopsis pseudotsugae is now known to occur throughout Great Britain and Ireland. It was observed in Denmark by Boyce (1925), by Wilson (1926), and by Hahn (1927). Hahn (44) also collected the fungus in Norway and Sweden. In 1926 Wilson collected it in Holland. For practical purposes the Douglas fir parasite may also be considered as being present in France, Germany, and Belgium; for the presence of the organism in these countries is strongly suspected, although collections have not as yet been made and identified.

SUMMARY

An important disease causing canker and die-back of the Douglas fir has recently been described in Great Britain and attributed to *Phomopsis pseudotsugae.*

Certain investigators have regarded this fungus as synonymous with the older species of *Phoma pitya* and *P. abietina* which have been considered as being the cause of various diseases of the Douglas fir and other conifers. There also has been considerable confusion in mycological and forestry literature concerning the identity of these older species.

Critical investigation has shown that the three organisms are distinct. *Phoma pitya* is considered a species of Sclerophoma and has been named provisionally *Scl. magnusiana. Phoma abietina* is regarded as *Phomopsis abietina.*

Sclerophoma magnusiana is regarded as a parasite occurring on *Pinus sylvestris*. Typical *Phomopsis abietina* has been recorded only from Germany and France on Abies, where it is considered to be the cause of a girdling disease of small twigs and branches. *P. pseudotsugae* has been shown by preliminary inoculation experiments to be a true parasite.

Phomopsis pseudotsugae is regarded as an Old World species present on *Larix europaea, L. sibirica, Abies pectinata, Cedrus atlantica* and *C. deodara*. It was collected by Rostrup in 1892 on *Abies pectinata*, but identified at that time as *Phoma pitya*. It is now known to occur also on *Pseudotsuga douglasii, P. douglasii* var. *caesia, P. glauca, Larix leptolepsis*, and *Sequoia gigantea*.

The present known distribution of *Phomopsis pseudotsugae* includes Great Britain and Ireland, Holland, Denmark, Norway, and Sweden. For practical purposes, it must be considered to be also present in France, Germany, and Belgium. The Douglas fir parasite is not known to occur in North America.

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SEPTORIA ACICOLA AND THE BROWN-SPOT DISEASE OF PINE NEEDLES

GEORGE G. HEDGCOCK[1]

A serious needle blight of species of pine has been known in the southern United States for more than ten years and has been present at least since 1876. The increased interest in forestry in recent years has resulted in the discovery of its importance. It attacks most severely the needles of longleaf pine, *Pinus palustris* Mill., but is not confined to this species. The loblolly pine, *Pinus taeda* L., slash pine, *Pinus caribaea* More., and other species often suffer from it. The writer has made a study of this disease, but is unable to complete the work and will now publish the results to date for the use of others who may investigate it in the future.

This disease was first called by the writer the "red spot" needle disease in his notes and correspondence, and this name is used by Dearness (5) but it is now known by the more appropriate name "brown-spot needle disease" or "brown spot," because brown spots on pine needles are the most conspicuous symptom of its presence. It has also been called by others "rust" or "leaf rust" (2, p. 26–28) but these names, if used, will lead to confusion, since they already have been applied to the type of disease caused by forms of Peridermium belonging to the genus Coleosporium.

The first symptom of the disease is the appearance of a small, light gray-green, irregularly circular spot which changes quickly to a brown color and later encircles the needle in a narrow band, finally killing that portion beyond the point of attack. The dead portions assume a lighter brown color. In severe forms of the disease many brown spots form on each needle throughout its length, often killing it during the first season after infection. Each successive season's growth of needles is attacked, with the result that the trees are nearly defoliated every year and either die or are greatly retarded in their growth.

Chapman (2) reports greater losses from the "brown spot" in longleaf pine than from spring fires. He found 5.3 per cent of the seedlings up to six inches in height killed by the disease, 34.3 per cent of those from six inches to two feet, and 4.5 per cent of those two feet and over. He asserts that a defoliation of the trees for three successive seasons kills them. The writer has observed the same effect in many localities in Georgia, Florida, Alabama, Mississippi, Louisiana, and South Carolina. The disease is much more severe on trees in grassy, unburned tracts, a fact also noted by Chapman (2). After the trees are five feet or over in height, it causes only

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slight injury. Edgerton and Moreland (7) in 1923 reported a severe blight of longleaf-pine seedlings in Louisiana, which from their brief description is similar to and no doubt identical with the brown-spot disease.

ISOLATION OF CAUSAL ORGANISM

The pine-needle fungus, *Septoria acicola* (Thüm.) Saccardo, is constantly found fruiting on the older brown spots and on the older dead brown portions of the diseased needles. No other species has been found in constant association with the disease. Dilution cultures of *Septoria acicola* were made from pycnospores isolated from needles diseased with the brown spot and were grown on several kinds of agar media. Heavy, coal-black, stromatal areas were formed on the mycelium in cultures. Upon these stromata a crowded layer of coal-black subspherical pycnidia were found, which were typical of the genus Septoria. These pycnidia contained numerous smoky gray cylindric to curved spores, which at first were nonseptate but later developed from one to three septa, three septa being typical for the fungus. None of these spores were found to be viable, and all failed to germinate in poured plates. The fungus was propagated readily, however, by transferring bits of the mycelium from growing cultures to poured plates or slant tubes of agar media.

INOCULATION TESTS

Two sets of inoculations were made with *Septoria acicola* in the greenhouses at Washington, D. C. The first was made February 14, 1923, on the needles of healthy potted pine trees as follows: 4 *Pinus caribaea,* 2 *P. palustris,* and 2 *P. taeda* were inoculated in moist chambers of an iceless refrigerator (8) by laying on the trees in contact with the living needles, dead needles of *Pinus palustris* collected at Urania, Louisiana, January 27, which were badly diseased with the brown spot and heavily infected with *Septoria acicola* in fruiting condition. The trees were lightly sprayed with water and left for two days. Some of the needles of the 2 *P. caribaea* and 2 *P. palustris* bore ragged gray-green spots March 27, which browned lightly by June 2. On October 13 a few of the needles were dead or partly dead and of a light brown color. These bore fruiting bodies of *Septoria acicola.* Control trees remained healthy.

A second set of inoculations was made July 26, 1924, from spore-dilution cultures of *Septoria acicola* isolated from the needles of *Pinus palustris* diseased with brown spot, collected at Urania, Louisiana, January 7, 1924. Eighteen healthy potted trees of *P. palustris* were used; six trees were sprayed with the exuded spores of the fungus in a water suspension, six were sprayed with a maceration of the stromata and pycnidia in water suspension, and six control trees were sprayed with sterile water. All

were placed in the moist chambers of iceless refrigerators for two days. The six trees sprayed with the spore suspension and the six control trees remained healthy. Some of the needles of each of the six trees sprayed with the maceration suspension became diseased, showing brownish-gray spots in September, 1924, and by February 25, 1925, had either wholly or partly browned and died. The negative result with the spore suspension indicates that the spores were not viable. The positive result with the maceration suspension may indicate infection either from the mycelium or from the presence of viable spores remaining in the pycnidia. The brown spots produced were not quite typical of those produced in nature, but the blighted leaves were fairly typical and of a light brown color. The effect of the strong sunlight in natural infections probably tends to cause more intense browning than greenhouse light which enters through white-washed glass and has an average intensity of about one-fifth of full sunlight. *Septoria acicola* was isolated from the diseased leaves from both experiments and grown in pure cultures from a spore dilution. The results of these experiments indicate that *Septoria acicola* is probably the cause of the brown-spot disease and that the fungus is probably parasitic.

TAXONOMY OF CAUSAL ORGANISM

De Thümen (13) described *Cryptosporium acicolum* Thüm. from a specimen collected by H. W. Ravenel at Aiken, South Carolina, in 1876 on *Pinus variabilis* Lamb. *P. variabilis* is a synonym of *P. echinata* Mill. There is a specimen in the Mycological Collections of the Department at Washington, D. C., collected by H. W. Ravenel (No. 1484, Mycotheca Universalis) on *Pinus variabilis* in 1876, which may or may not be a co-type. The pine, however, is not *P. echinata* but *P. caribaea* More. Saccardo (11, p. 507) in 1884 changed the name of *Cryptosporium acicolum* Thüm. to *Septoria acicola* (Thüm.) Sacc. on account of the septate spores. Geo. Martin (9) in 1887 reported the fungus on pine needles (Ravenel's collection ?) from South Carolina under the name *Septoria acericola.*

G. H. Martin (10) reported the brown-spot disease from Florida in 1922 on *Pinus taeda* and in 1923 from Louisiana on *Pinus palustris.* A published article in 1920 based on Hine's investigations (1), incorrectly attributed the brown-spot disease to *Lophodermium pinastri* (Schr.) Chev., which fruits on dead pine needles and occurs rarely on longleaf pine and only on dead fallen needles. *Lophodermium australe* Dearness is common but sparse on old gray needles of this pine clinging to the branches but is not associated constantly with the brown-spot disease. Chapman (2) in 1926 ascribed the disease to *Septoria pini* which is not known to occur on longleaf pine. A description of *Septoria acicola* follows:

Septoria acicola (Thüm.) Sacc. Syl. Fung. 3: 507, 1884.

Syn.; *Cryptosporium acicolum* Thüm. Fungi Am. Trig. sp. nov. Flora 61: 178, 1878 (13).

Actinothyrium marginatum Sacc. Nuov. Giorn. Bot. Ital. 27: 83, 1920 (12).

Lecanosticta pini Syd. Ann. Myc. 20: 211, 1922 (12).

Lecanosticta decipiens Petr., Ann. Myc. 20: 214, 1922 (12).

Lecanosticta acicola (Thüm.) Syd. Ann. Myc. 22: 400, 1924 (12).

Acervuli amphigenous, globose at first, 50–100 μ in diameter, becoming elongate up to 1.5 mm., seated in the mesophyll, discharging spores through a cleft in the epidermis. Basal layer of colored compact pseudoparenchyma.

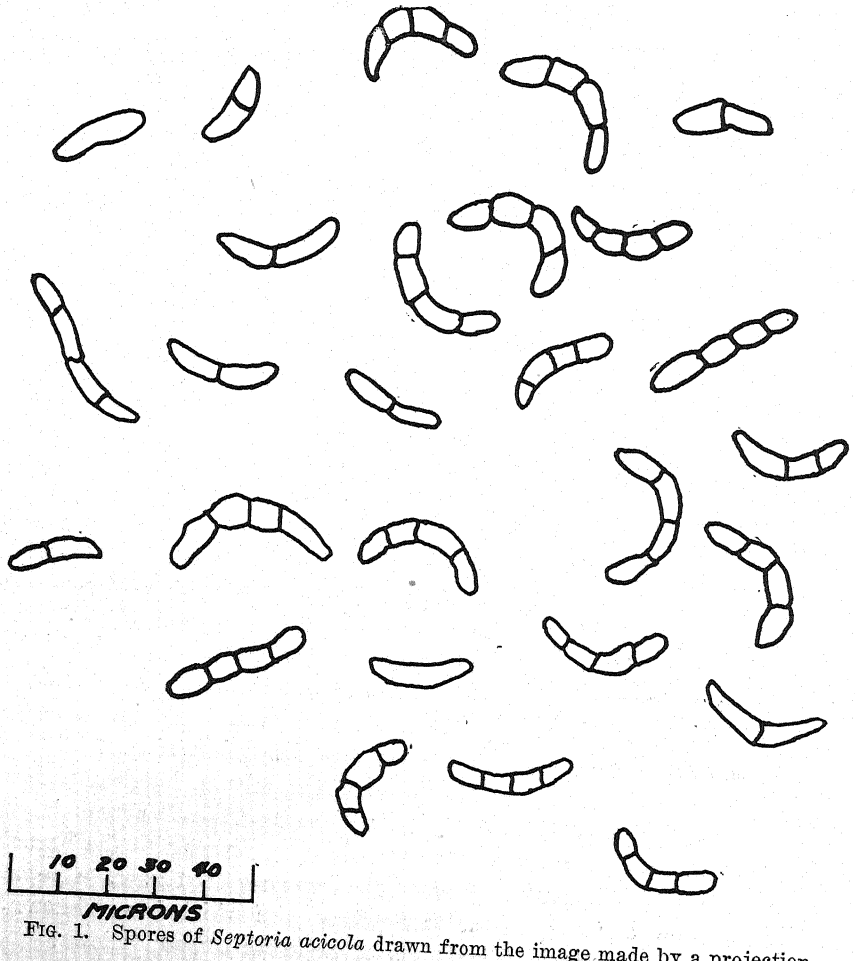


FIG. 1. Spores of *Septoria acicola* drawn from the image made by a projection apparatus.

Spores simple, subhyaline, becoming 1–3 septate and smoky, often curved more in one half than the other, mean of 100 spores 29.5 by 3.8 μ with a sextile range[2] of 32–27 by 4–.5 μ (Fig. 1). The acervuli occur on brown spots forming on living pine needles and also on other lighter brown portions of dead needles.

On the needles of *Pinus palustris,* F. P. No. 41664, collected by Freda Detmers at Bogalusa, Louisiana, January 7, 1924.

Sydow and Petrak (12) have some basis for putting *Septoria acicola* in the genus Lecanosticta, since it differs in its natural form of fruiting from typical species of both Cryptosporium and Septoria. In artificial cultures, however, it resembles closely species of Septoria and the writer chooses at present to leave it in the genus Septoria.

Dearness (4) has found an ascus-bearing species, *Oligostroma acicola* Dearn., closely associated with *Septoria acicola* in a number of southern collections made by the writer and considers it to be probably a perfect stage of the latter fungus. Cultural proof, now lacking, will be necessary to establish this relationship.

HOST RANGE AND GEOGRAPHICAL DISTRIBUTION

In the collections of the Office of Forest Pathology at Washington, D. C., there are 121 collections of *Septoria acicola* and the brown-spot disease on ten species of pine from 11 States, and in the Mycological Collections of the Department there are 11 more collections with two additional host species. The number of collections on each species of pine for each State is now given.

On *Pinus attenuata* Lemm., Ore. 1.

On *P. caribaea,* Fla. 4, Miss. 1, and S. Car. 1.

On *P. echinata* Mill., N. Car. 2, and S. Car. 1.

On *P. glabra* Walt., Fla. 3.

On *P. nigra austriaca* Schneid., Kan. 1, Ky. 1, and Mo. 1.

On *P. palustris,* Ala. 2, Fla. 41, Ga. 8, La. 13, Miss. 5, N. Car. 2, S. Car. 9, and Tex. 1.

On *P. ponderosa* Laws., Idaho 3.

On *P. rigida* Mill., N. Car. 2, Pa. 1, Tenn. 1, and Va. 1.

On *P. serotina* Michx., S. Car. 2.

On *P. sondereggii* Chapm. (*P. palustris* × *P. taeda*), La. 1.

On *P. taeda,* Ark. 1, Fla. 5, Ga. 1, La. 2, Miss. 1, N. Car. 1, S. Car. 6, Tenn. 3, and Tex. 1.

On *P. virginiana* Mill., Ga. 1, and N. Car. 2.

From the data given, it will be seen that the present known range of *Septoria acicola* and of the associated brown-spot disease in the eastern United

[2] The "sextile range" is that remaining after the sixth of the spores that are smallest and the sixth that are largest have been discarded.

States is from Pennsylvania west to Kansas and south to Florida and Texas, and in Oregon and Idaho in the northwest.

CONTROL OF THE DISEASE

Fire destroys the spores of *Septoria acicola* and must to some extent hinder the spread of the disease; at the same time, however, it kills many of the small pine trees. The effect of the fungus versus the effect of fire is still a subject of controversy among foresters. It is not advisable to recommend the burning of the ground cover of areas with diseased pines for the purpose of destroying the spores of the fungus until the facts are more fully known. The subject of the effect of fire in controlling the disease has been discussed in a number of articles by Chapman (2, 3), Hine (1), Demmon and Hadley (6), and Wyman (14). A few data from experiments have been published (1, 2, 3, 14) but the results are too limited in extent to form a basis for a final conclusion. Experiments from a number of localities widely separated and under varying conditions will be necessary to finally ascertain the correct method of handling diseased areas.

The use of fungicides for the control of the brown-spot disease in forest-tree nurseries is advised. The following method, suggested by the writer, was used in young stands at Bogalusa, Louisiana, in the spring of 1928. Spray every two weeks with a spraying outfit that gives a misty spray, beginning when the young needles emerge and ceasing when the leaves have attained their growth. Use standard Bordeaux 4–4–50 with the addition of two pounds of either whale-oil soap or a casein soap like "Kayso" to every 50 gallons of spray solution. Ordinary Bordeaux without the soap rolls off pine needles without adhering to them. The use of Bordeaux and of lime-sulphur sprays in this manner at Bogalusa controlled the disease as long as the spraying was continued. The period suggested by the writer was too short. Spraying should continue longer.

Foresters, plant pathologists, and others interested in the brown-spot disease are requested to send specimens to Paul V. Siggers, Associate Pathologist, Southern Forest Experiment Station, New Orleans, La. Mr. Siggers will continue the study of this disease.

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THE RELATION OF LIME-SULPHUR SPRAYS TO THE ABSCISSION OF YOUNG APPLES

F. S. Howlett and Curtis May

Strong lime-sulphur solutions, sprayed on young apple foliage and fruits, may cause marginal burning of the leaves, cupping of the blades, curvature of the midrib, burning out of scab spots, and deformity of the young apples. This type of injury has been recognized for a long time. Recently, it has been stated that excessive abscission of young apple fruits may be caused by lime-sulphur spray, even though there is no visible spray injury on the leaves or fruit.

Bagenal and others in England (1) concluded from their spray experiments in 1924 that Bordeaux mixture 4–4–50 did not increase the fruit drop from a healthy tree, but that lime-sulphur 1–29 induced an excessive amount of it. They state that there was no serious spray injury of leaves or fruit visible to the unaided eye. They add that "the unusual percentage of drops due to spraying with these two fungicides cannot be exactly determined from evidence available, owing to the variation shown by the control trees."

Dutton's (2) experiments were largely concerned with the effect of lime-sulphur and Bordeaux sprays on the set of fruit when some leaf fall occurred. He stated that the June drop was excessive for trees sprayed very heavily with lime sulphur but was normal for the Bordeaux-sprayed trees.

The present study was designed to determine the effect of the second after-bloom spray on abscission of young fruits when there were no visible marks of spray injury either on them or the foliage.

Method

The trees used were healthy, moderately pruned, 27-year-old Ensee and Grimes Golden varieties growing in the grass-mulch orchard of the Ohio Agricultural Experiment Station at Wooster. Each was given an annual pre-bloom application of 5 pounds of nitrate of soda. These varieties were selected, not only because they are susceptible to spray injury, but also because they bear flowers on shoots growing sufficiently long to be easily bagged or individually sprayed.

Three pre-blossom sprays and a calyx spray were applied in 1927. The following season the trees were dusted up to and including the calyx application.

Liquid lime sulphur at dilutions of 1 to 15, 1 to 40, and 1 to 60 was tested the first season. The following year liquid lime sulphur, 1 to 60, 1 to 100, and 1 to 120, and Bordeaux mixture, 2–4–50 and 1–2–50, were used.

After the fruits had passed through the first drop the number of fruits in each cluster was reduced to two laterals (Fig. 1). Those left on the clus-

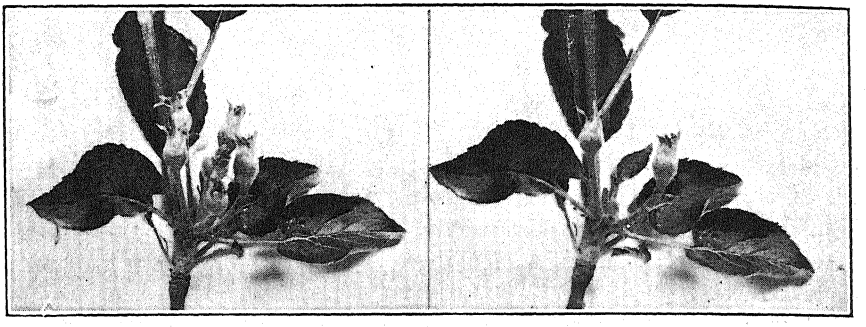


FIG. 1. Left, cluster of Ensee before thinning, 1928. Right, same cluster after thinning to 2 laterals.

ters showed every evidence of having successfully passed the first wave of dropping. The elimination was done each year by one person and over the various limbs an attempt was made to alternate the clusters receiving the various treatments.

In 1927, the clusters were sprayed individually by means of a hand-barrel pump. A galvanized-iron pan was made with a hole in the center of the bottom. This hole could be closed by a crescent-shaped slide. There was also a reservoir underneath to catch the drip. Before spraying the cluster was placed through the opening of the pan, after which the hole was almost completely closed by means of the slide. The cluster was then well drenched with spray. The drip was retained by the reservoir.

In 1928, approximately 750 fruit clusters were selected on each tree and the fruits reduced, as before, to two per cluster. These clusters were divided into three sets and the sets marked by labels of different colors. Two sets of clusters were covered with glassine bags. The tree was then sprayed with the regular orchard sprayer (375 lbs. pressure) using the stronger dilution of lime sulphur. As soon as the selected clusters of Set 1 had dried they were bagged. The bags were then removed from Set 2, and the tree again sprayed with a weaker solution. The bags were then removed from the clusters of Sets 1 and 3. Two spray dilutions and an unsprayed check were obtained on each tree by following this procedure. In both years the elimination, bagging, and experimental spraying were completed within 24 hours. The data were taken shortly after the June drop was completed.

RESULTS

The results of the tests of 1927 are given in table 1. It is to be observed that the percentage of fruit set of all the sprayed clusters of both trees was

TABLE 1.—*The effect of lime sulphur on abscission of young Ensee apples. Wooster, 1927*

Tree	Dilution of spray	No. of clusters	Percentage set of fruits	Percentage of clusters with		
				0 fruit	1 fruit	2 fruits
411–3	1–15	93	51.6	22.6	51.6	25.8
	1–40	96	60.4	16.7	45.8	37.5
	1–60	89	60.7	16.8	43.8	39.4
	unsprayed	102	84.8	4.9	20.6	74.5
411–2	1–15	82	61.0	17.1	43.9	39.0
	1–40	94	68.1	10.6	42.6	46.8
	1–60	100	67.0	16.0	34.0	50.0
	unsprayed	99	93.4	0.0	13.1	86.9

lower than that of the unsprayed clusters. In order to obtain the odds that the differences found were not due to chance the variability of the determinations was measured by dividing the samples at random into sub-samples of approximately 50 clusters each. The deviations of these sub-samples from their respective sample means were squared. From these values t, as given in Fisher (3), was computed for the various differences. The values of t were converted into odds by the use of Student's tables (5).

Combining the data of the two trees the odds that the differences obtained in 1927 between the clusters sprayed with 1 to 60 solution and the unsprayed

TABLE 2.—*Effect on fruit abscission resulting from spraying young apples with lime-sulphur or Bordeaux mixture. Wooster, 1928*

Apple variety	Fungicide	No. of clusters	Percentage set of fruits	Percentage of clusters with		
				0 fruit	1 fruit	2 fruits
411–1 Ensee	Lime sulphur 1– 60	222	73.4	5.9	41.4	52.7
	" " 1–100	223	76.9	5.4	35.4	59.2
	Check	215	83.3	3.6	26.1	70.3
409–4 Grimes	Lime sulphur 1– 60	174	31.9	48.8	38.5	12.6
	" " 1–100	187	51.3	20.9	55.6	23.5
	Check	216	48.1	27.8	48.1	24.1
409–5 Grimes	Lime sulphur 1– 60	172	59.4	17.1	46.8	36.1
	" " 1–120	169	63.7	12.8	47.1	40.1
	Check	195	69.0	10.3	41.5	48.2
409–7 Grimes	Bordeaux 2–4–50	222	55.2	16.8	56.2	27.0
	" 1–2–50	222	46.8	26.7	51.1	21.1
	Check	224	58.2	22.3	48.2	29.5

clusters were not due to chance are 332:1.[1] There were no significant differences between the clusters sprayed with the 1 to 40 and the 1 to 60 dilutions.

The data for 1928 are given in table 2. Ensee tree 411–1 is a companion tree of those used in 1927. The data indicate that the 1–to–60 (odds 1000:1) and the 1–to–100 (odds 155:1) dilutions caused slight fruit abscission on this tree.

On Grimes Golden tree 409–4 there was a marked reduction (odds 908:1) from the clusters sprayed with 1 to 60, but none from those sprayed with 1–to–100 lime sulphur. On tree 409–5 the odds that the set reduction caused by 1–to–60 was significant were 9.05:1. The 1–to–120 dilution did not cause a significant loss.

If the data from these three trees are combined and the odds calculated, the results are as follows:

1–to–100 and 1–to–120 vs. unsprayed	4.8:1
1–to– 60 vs. "	832:1
1–to–60 vs. 1–to–100 and 1–to–120	184:1

It is apparent that 1–to–60 lime sulphur reduced and that 1–to–100 or greater did not reduce the set of fruit on these trees. However, there was a significant difference between the 1–to–60 and the 1–to–100 dilutions. Since the former caused fruit abscission and the latter did not, we may conclude that the dilution at which there would cease to be significant injury would be between these two dilutions.

The loss of fruit due to 1–to–40, 1–to–60, or greater dilutions of lime sulphur was not great enough to affect their normally heavy crop. If trees of these two varieties are of the usual commercial vigor they have a very heavy set, even after the June drop is completed, and thus require considerable thinning.

The results from the tree sprayed with Bordeaux were inconclusive. Because of the heavy rains subsequent to the application of the spray, there were a considerable drip of Bordeaux and a consequent russeting and deformity of many of the fruits of the check clusters.

The great extent to which the second, or June, drop was reduced in Ensee on the unsprayed clusters by reducing the fruits to two is indicated by the relatively high percentage sets (Table 1) of the check clusters, 84.8 and 93.4 per cent, respectively. The fruit drop was not so greatly reduced on Grimes Golden as on Ensee. However, unpublished data of the senior writer indicate that the percentage of fruit retained by the treated clusters was greater than that of clusters whose fruits were not reduced before the June drop.

[1] The writers wish to thank Mr. Marion T. Meyers for his assistance in the statistical treatment of the data.

DISCUSSION

There is a well-known correlation between lime-sulphur and Bordeaux spray injuries and the weather conditions at the time of and immediately following the application of the spray. The maximum and minimum temperatures for the two weeks following the application of the experimental sprays are given in table 3.

TABLE 3.—*Maximum and minimum temperatures (Centigrade) for 2 weeks following the application of the experimental sprays. Wooster, 1927, 1928*

	May, 1927						June, 1927							
	26	27	28	29	30	31	1	2	3	4	5	6	7	8
Max.	22.7	16.1	15.0	18.8	20.0	27.7	21.1	16.1	17.7	20.0	25.0	16.1	17.7	20.0
Min.	12.7	6.6	3.8	3.8	10.0	3.8	6.1	3.8	2.7	8.8	17.2	6.6	7.7	5.0

	June, 1928													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Max.	21.1	22.7	18.3	20.0	22.7	20.5	19.4	18.8	10.0	20.5	17.2	20.0	25.5	28.8
Minn.	4.4	8.3	9.4	2.7	11.1	12.2	10.0	10.0	17.7	11.1	7.2	5.5	10.0	20.5

On none of the days on which sprays were applied was the temperature high enough to cause visible fruit or foliage injury when the standard strength of lime sulphur was used. It is generally regarded that 26.5° C is the danger point for lime-sulphur-spray burn. In 1927 it was five days, and in 1928 two weeks after the spray was applied, before this temperature was recorded.

In general, the weather during the periods following the spraying in both years was cool. Cool weather is supposedly not conducive to lime-sulphur injury. Bordeaux is, however, particularly injurious in cool, wet weather. Such a period followed the spray applications of 1928 and severe russeting and dwarfing of the fruit occurred on the Bordeaux-sprayed trees.

The data given by Bagenal (1) and Dutton (2) indicate that they consider the second after-bloom spray to be the one responsible for excessive abscission of apple fruits. This, the writers believe, is due to the fact that the spray is applied usually, if not always, just previous to or during the June drop of the varieties employed in the experiment. The data in table 4 indicate that this is the case at Wooster.

Each year normal dropping continued for two to three weeks after the application of this spray. The writers wish to point out that mere observa-

TABLE 4.—*Relation of the time of after-bloom sprays to end of fruit abscission. Wooster, 1924–1928, inclusive*

Year	Dates of application		Date of period of June drop
	Petal-fall spray	Two-weeks spray	
1924	May 22–29	June 9–13	June 6–July 7
1925	May 8–16	May 26–June 4	June 4–July 1
1926	May 26–31	June 10–17	June 10–July 7
1927	May 12–17	May 31–June 2	June 2–July 1
1928	May 25–29	June 8–13	June 9–July 1

tion of fruits falling during or after either the calyx or 2-weeks' spray is not, in itself, evidence that the abscission is due to spray injury.

The relation of varietal differences in fruit-setting characteristics to food competition among the fruits of a cluster deserves consideration in a study of the relation between spray injury and abscission (4). Immediately after petal fall on Ensee and Grimes Golden 3 to 5 flowers in a cluster usually enlarge and survive the first drop (figure 1). The enlargement is marked usually about a week before the normal time of application of the second after-bloom spray (2–3-weeks' spray). Varieties like Ensee and Grimes Golden which pass through the first drop with a heavy set commonly have a heavy June drop. This drop appears to be due largely to food competition and unless some means are taken to control this factor, it would appear to be very difficult to obtain reliable data on this subject. The extent of the reduction of the June drop by reducing the fruits to 2 per cluster has already been pointed out. It is highly probable that the differences between the set of fruit on the sprayed and unsprayed clusters were due to the materials used and not to loss of fruit because of insufficient food supply.

Trees of the same age and apparently the same vigor may vary in their ability to hold their fruit. This is shown by the data (Table 2) for the unsprayed clusters of Grimes Golden trees 409–4 and 409–7. Both trees appeared to be of equal vigor but they had June drops of different degrees of severity. Spraying one tree and not another and comparing the drops from each, and assuming that differences obtained are due to the spray treatment, are open to criticism. Tree variation is such an important factor that the spraying of a few trees and comparing them with unsprayed trees are rather unsatisfactory procedures.

The writers make no attempt to apply the results of this study to any but the varieties used. Ensee and Grimes Golden are susceptible to spray russeting of the fruits and possibly are more affected by lime-sulphur solutions than other heavy-setting varieties.

SUMMARY

1. Lime-sulphur and Bordeaux mixture were applied as second after-bloom sprays to determine whether they caused excessive fruit abscission when there was no visible foliage injury.

2. The number of fruits in the individual clusters was reduced to two to eliminate food competition as a factor in fruit abscission.

3. The different spray dilutions were applied to the same tree to eliminate individual tree variation.

4. Lime-sulphur solutions of 1–40 and 1–60 dilutions caused some abscission of fruit from Ensee and Grimes Golden trees.

5. Lime-sulphur solutions of 1 to 100 or greater dilution did not cause significant abscission of fruit from Ensee and Grimes Golden trees.

6. The abscission due to any of the spray dilutions was not sufficiently great to reduce their normally heavy commercial crops.

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IS THE ASTER–YELLOWS VIRUS DETECTABLE IN ITS INSECT VECTOR?

IRENE D. DOBROSCKY

The leaf hopper, *Cicadula sexnotata* Fall., has been shown to be the specific carrier of the virus of aster yellows (6). Furthermore, this virus must remain in the body of the insect for at least ten days before it can reinfect another plant. Similar conditions have been found to obtain in the case of curly top of sugar beets and the leaf hopper, *Eutettix tenellus* (Baker) (8); and, also, in the case of streak of maize and the leaf hopper, *Balclutha mbila* Naude (9). The necessity for an incubation period of the virus would seem to indicate that we are dealing with an organism which must either multiply in the insect or undergo a definite part of its life cycle there. This simulates closely the facts known about malaria, yellow fever, Texas cattle fever, nagana disease of cattle, and African sleeping sickness.

Because of the long incubation period of the aster-yellows virus in its insect vector it was thought this leaf hopper might be a favorable one in which to detect the virus. An intensive study of the morphology and cytology of the insect was therefore undertaken. Such a study might result in the discovery of the causative agent of yellows or some evident reaction of the insect to the virus. It might result even in the discovery of an intracellular body associated with the virus in the insect.

Botanists who have studied the tissues of plants affected with virus diseases have found intracellular bodies in connection with many of these diseases. At least twenty-eight species of plants having the mosaic type of virus disease have been reported as containing these so-called "x-bodies," or inclusion bodies.

Plants affected with the yellows type of virus diseases, such as aster yellows, peach yellows, curly top of sugar beets, and cranberry false blossom, have not been found to contain intracellular bodies. The morphological symptoms of a plant affected with aster yellows are, however, very striking and characteristic. Nevertheless, no bacterium, protozoan, or fungus has been found associated with the disease. The causative agent has not been found in those plants susceptible to the aster-yellows virus. It seemed advisable to search for it in the insect.

METHODS

The histology and cytology of the leaf hopper, *Cicadula sexnotata,* were studied by making smears and examining these under dark-field illumination, by gross dissections of the alimentary tract, and by serial paraffin sections.

Smears. In making smears about fifty adults, hatched and reared on diseased asters, were used. Similar virus-free adults served as checks. Each insect was slowly crushed between a slide and a cover slip and, while the smear was still wet, it was examined under dark-field illumination. Numerous globules of fat were observed, some showing Brownian movements. There was no indication of vital movement shown by any of the bodies in the smears.

When the smears had dried they were stained with Wright's blood stain for one minute. All the slides showed small brownish granules which were the pigment granules of the compound eyes. About one-half of the smears contained oval-shaped bodies 8 μ to 12 μ long, with pink capsule-like rims, blue cytoplasm, and bright red nuclei. A few forms were found dividing. These bodies resemble the spores of Cladosporium, a soil-infesting fungus. As the spores were never found in the paraffin sections, it is probable that the insects bore them externally. Nuclei of all sizes and stages of activity were found in the smears. Crushed nuclei in the spireme stage took a pink stain and resembled clumps of bacteria. Many fragments of broken sperms were found but there was nothing present resembling either a bacterium or a flagellate.

Dissections. Gross dissections were made to study the alimentary canal. The stomach was found distended with a dark brown fluid. Continuing from the stomach is a narrower portion of the midintestine. This is very white and opaque in appearance, due to the accumulation of calcium-carbonate crystals within the cells. It is thought that the liquid food passes by osmosis from the stomach to the posterior intestine without passing through this narrow portion of the midintestine. Two of the most important appendages of the alimentary canal are the salivary glands and Malpighian tubules. The Malpighian tubules are four in number, rather large and thick. The salivary glands are in the head and prothorax. They are so small and so transparent that it is difficult to find them.

No cysts, lesions, or abnormal enlargements of any organ were found in the course of this macrodissection.

Paraffin Sections. More than a thousand insects, adults, and nymphs were fixed in various reagents, including Gilson's, Regand's, Bouin's, Flemming's weak, and Carnoy's solution. The sections were cut at 4 μ or 7.5 μ. They were then stained with Wright's, Giemsa's, Wolbach's Modified Giemsa, Heidenhain's Haemotoxylin, Flemming's, Mallory's Methylene blue, Pianese III B, Gentian violet, and Safranin, or acid fuchsin stain. For secretory cells of the salivary-gland type, Gilson's fixative followed by Wright's Romanowsky stain gave the best results. The Romanowsky stain, as put up by Coleman and Bell Co., proved most satisfactory. McNeal (7) has recently shown that there are four essential dyes in this stain. They are eosin, methylene blue, methylene azure, and methylene violet.

OBSERVATIONS

The parasites which cause malaria are found in cysts in the stomach walls and also as free swimming flagellates in the salivary glands of their mosquito hosts. Herpetomonas associated with oriental sore was found in the salivary glands of the fly *Phlebotomus papatasi* (1). Holmes (5) found *Herpetomonas elmassiani* (Migone) in the salivary glands of *Oncopeltus fasciatus* Dall., a bug which feeds on milkweeds. The flagellate, *Leptomonas davidii* Lafont, was found by França (4) in the salivary glands of the hemipteron *Stenocephalus agilis* Scop. which feeds on Euphorbias. These organisms are all ingested by their insect hosts. In several insect-borne diseases the causative organism has been found in the alimentary tract, proper. It seemed advisable, therefore, to make an intensive study of the salivary glands and the alimentary tract of the leaf hopper in question in the hope of finding some indication of the presence of the virus.

Salivary glands.[1] The salivary glands lie beneath the brain and occupy the dorsal floor of the head. They are made up of equal halves lying on either side of the œsophagus. Each half resembles a small bunch of grapes with a fore-shortened central stem. The lobules, twenty-three in each half, are large secreting cells containing two nuclei. Physiologically, the salivary glands may be divided into three types of cells, the mucous cells, the serous cells, and the cells which line the accessory or reservoir glands.

In making a comparative study of the salivary glands of healthy and viruliferous individuals, the following characteristics were noted: 1. Size, shape and staining properties of each lobule; 2. Texture and staining reaction of cytoplasm; 3. Vacuoles and inclusions in cytoplasm; 4. Size, staining properties and physiological state of nuclei; 5. Amount of secretion.

Though several hundred insects were examined with these points in mind, no constant difference was observed. No lesions, no cysts, no difference in size or shape of lobules, nor any alteration in the physiology of the cells, as indicated by their staining reactions, could be found.

After this study of the cytology of the secreting cells, the author proceeded to look for any foreign organism that might be present. The numerous stains used were calculated to detect the presence of bacteria, fungi, protozoa, Rickettsia or x-bodies. None of these was found in the salivary glands of either the normal or the viruliferous individuals.

In one experiment a series of adults, fed from one to twenty-three days on diseased asters, was studied in the hope of finding some progressive symptoms to account for the incubation period of the virus. No symptom indicative of the presence of the virus could be found.

[1] A more detailed morphological description of the salivary glands will be published by the author in another paper.

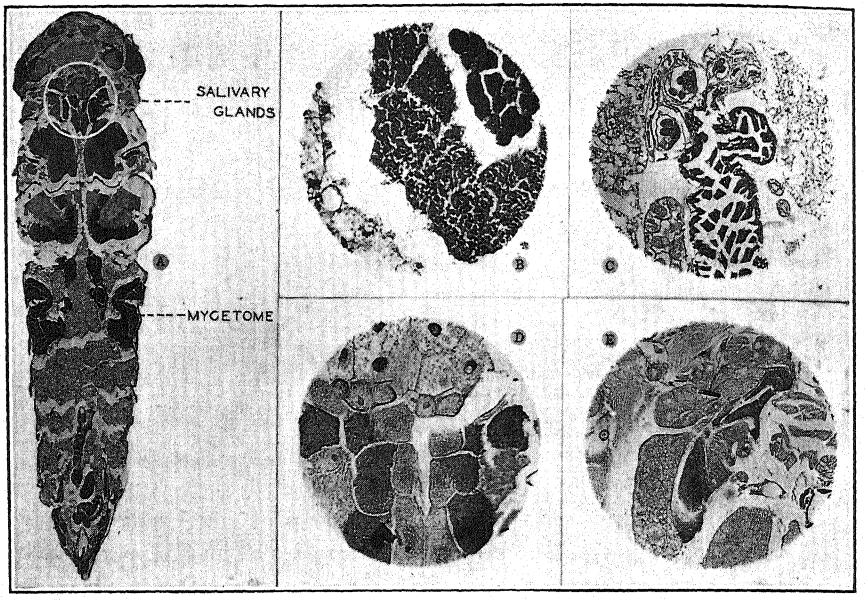


FIG. 1. A. Longitudinal section of adult female of *Cicadula sexnotata*. Shows salivary glands in prothorax and mycetome on either side of the first and second abdominal segments. (7 μ thick, × 50.)

B. Mycetome in detail. Note zone of empty cells with small marginal nuclei. (7.5 μ, thick × 430.)

C. Midintestine of disease-carrying insect. (7 μ thick, × 180.)

D. Salivary glands of adult insect starved ten hours. (7 μ thick, × 200.)

E. Lateral view of salivary glands of healthy normal insect. (7 μ thick, × 160.)

In another experiment some adults were starved and their salivary glands compared with those of normal insects in order to see how the cells were altered. It was found that the individual cells of the glands of starved insects were larger, due to an accumulation of secretion. This is illustrated in figure 1, D. Figure 1, E, shows the salivary glands of a normal healthy insect.

Alimentary tract. It is a well known fact that most insects shed their chitinous covering several times during the course of their development. The anterior and posterior intestines are lined with a thin intima, a continuation of the outer heavy chitin. This intima is shed along with the outer coat when the insect molts. An insect which has fed on a diseased plant and becomes infective does not lose its infective principle in spite of this process of shedding. Since the midintestine is not lined with intima, it would seem that this is the logical place to look for obligate parasites. Figure 1, C, is a photograph of the anterior portion of the midintestine, showing the large darkly stained cells which compose it.

It has been found in the case of several insect-borne diseases that the parasites are first localized in some part of the alimentary canal and from there get into the salivary glands. *Cicadula sexnotata* was found remarkably free from all parasites. Its alimentary tract does not harbor any foreign organisms.

Cowdry (3) examined one hundred and eleven species of insects and found that nineteen contained Rickettsia bodies. The writer, using Cowdry's methods of staining and fixing was unable to find any Rickettsia in the body of this leafhopper.[2]

Mycetome. The mycetome is an organ present in most Homoptera. This might easily be mistaken for a sign of the disease in the insect under discussion. Witlazil (11) regarded it as excretory in function, others ascribed to it a nutritive function. Sulç (10), Buchner (2), and Glaser, however, have concluded that the organ is parasitic.

The mycetome is a large paired organ located in the first two abdominal segments, as shown in figure 1, A. The cells composing it are closely packed with organisms of a fungal nature, whence the organ derives its name.

Three distinct zones, comprising different types of cells, can be seen in this organ. Figure 1, B, an enlargement of a portion of the mycetome, shows these zones very clearly. In the center of the organ are numerous compact cuboidal cells. The nuclei are almost one-half as large as the cell, and the cytoplasm is densely filled with a mycelium-like growth. With Wright's stain this zone is colored dark blue. The next zone is composed of very much larger cells with smaller nuclei. The cytoplasm is obscured by the presence of the thick mycelium-like strands. This zone stains a pinkish purple. The third zone is one-cell thick, the cells being almost empty of cytoplasm. Nothing but small, compact, dark blue nuclei are to be found along the borders of these outer cells.

The whole organ is embedded in the fat tissue and has no direct connection with any other organ. It is present in virus-free as well as virus-bearing individuals. The organisms within this mycetome are supposed to migrate to the eggs and are so transmitted from one generation to another. Since this organ is hereditary and since it is not in communication with any other organ, it is not likely that it can be concerned in the problem of the insect's ability to harbor the yellows virus.

DISCUSSION

The virus diseases of plants are essentially insect-borne. Sucking insects, especially aphids and leaf hoppers, are the chief disseminating agents

[2] Dr. Cowdry was kind enough to examine some of the writer's slides and confirmed this conclusion.

under natural conditions. Of the leaf hoppers known to be virus vectors, all but one are very closely related in the classification of insects.

Up to the present time, no one has done any intensive research on the morphology and cytology of these insect vectors with the view to locating the virus. The writer has been unable to find any histological or cytological differences between virus-free and virus-bearing individuals of the species *Cicadula sexnotata*. While the tecnique used has given only negative data, it is possible that other methods of fixing and staining may yield positive results.

A study of the other insect carriers of plant viruses may give a clue as to where the yellows virus may be located in the body of the insect. Physiological studies may also throw light on the subject. Significant results might be obtained by making determinations of the pH of individual insects. If certain portions of virus-carrying individuals were isolated and healthy insects made to feed on them, one might get an indication of the whereabouts of the virus. Several attempts have been made to culture the virus in plant decoctions. Some experiments in culturing the virus in insect juices should also be undertaken.

SUMMARY

A study of smears of virus-bearing insects under dark field illumination, of gross dissections, and of paraffin sections did not reveal any visual evidence of the presence of the aster-yellows virus in *Cicadula sexnotata*.

The mycetome, a hereditary parasitic organ, is evidently not concerned in the problem of the virus-carrying ability of the insect.

After an intensive study of the salivary glands and alimentary tract of *Cicadula sexnotata* with a view to finding the causative agent of aster yellows, the writer was unable to find such an agent or any lesions which might be due to the presence of such an agent. No bacterium, Rickettsia, fungus, protozoan, or x-body was found which could be considered of etiological significance.

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LIFE HISTORY AND PATHOLOGICAL ASPECTS OF GODRONIA CASSANDRAE PECK (FUSICOCCUM PUTREFACIENS, SHEAR) ON CRANBERRY

C. L. Shear and Henry F. Bain

End rot is now known as the most important fruit rot of cranberries. The pycnidial form of the fungus causing end rot was described by the senior writer in 1917 as *Fusicoccum putrefaciens* (5), and it was suggested that a Discomycete, occasionally associated with the pycnidia about the blossom end of mummied berries, was perhaps the ascogenous stage of the fungus. The earlier studies of the end-rot organism were confined to the eastern United States, where ascocarps of the Discomycete were but rarely found and viable ascospores were difficult to obtain.

B. A. Rudolph succeeded in producing typical pycnidia of Fusicoccum from cultures made from the tissues of an immature apothecium of this Discomycete found on a decayed berry in Massachusetts in 1917. Three years later the senior writer was able, through the assistance of Rudolph, to obtain some fresh ascocarps from the Pacific Coast, but was unable to obtain single-spore cultures. Cultures made by transplanting asci and paraphyses after careful surface sterilization all showed the lemon-yellow to orange-citrine color characteristic of Fusicoccum as illustrated in the colored plate accompanying the first description (5), and several cultures finally produced typical pycnidia of the fungus.

While carrying on cranberry-disease investigations in Oregon and Washington in 1923, the junior writer found an abundance of mature apothecia with viable ascospores. Twenty single-ascospore cultures were made, partly from apothecia on berries and partly on stems. Germinating ascospores are shown in figure 6, B. Germ tubes most frequently arise from the terminal cells of the spores, although it is not uncommon for one or more intermediate cells to produce germ tubes simultaneously. These cultures all showed the typical mycelial color and general appearance and finally produced typical pycnidia of *Fusicoccum putrefaciens*. No apothecia, however, have ever developed in any of these or other single-ascospore or pycnospore cultures. The evidence already given, however, seems sufficient to prove the genetic relation between the two forms of the fungus.

After many attempts to identify the apothecial form of the organism by comparison with various Discomycetes which have been found on Vaccinium and closely related Ericaceous plants, it was finally found to agree with *Godronia cassandrae* Peck (2, p. 50), which was described from specimens collected on dead branches of *Cassandra calyculata* L. at Karner, N. Y., in August, 1885. The senior writer recently found excellent specimens of this

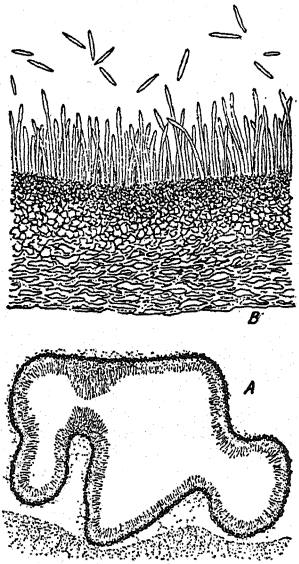


FIG. 1. *Fusicoccum putrefaciens*: A, Vertical section of a chambered pycnidium from a corn-meal agar culture, X 43.5; B. part of section of pycnidium grown in pure culture on sterile sweet clover stem (*Melilotus alba*), showing sporophores and spores, X 420. After Shear.

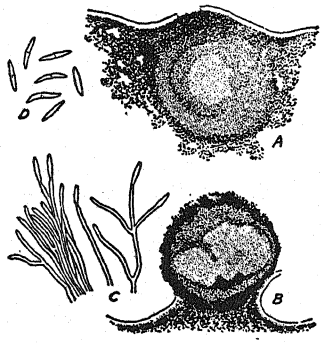


FIG. 2. *Fusicoccum putrefaciens*: A, Vertical section of a young pycnidium embedded in the tissue of a recently rotted cranberry; B, vertical section of a nearly mature pycnidium showing two chambers, X 43.5; C, separate branched and simple sporophores with immature spores; D, separate spores, showing variations in form and size, X 420. After Shear.

fungus in both stages on Cassandra at Upper Saranac and Schoon Lake, N. Y., and Lakehurst, N. J. This material agrees with that found on both the fruit and dead stems of the cranberry and also with Peck's original description of *Godronia cassandrae* which is, for convenience, quoted in full below:

GODRONIA CASSANDRAE N. SP. PECK

Receptacle small, .02 to .03 inches [.5 to .7 mm.] broad, sessile or nearly so, depressed, urceolate, tawny brown, the hymenium whitish or livid when moist, darker when dry, the narrow mouth entire or slightly dentate-lacerate, almost closed when dry; asci cylindical, .004 to .005 inches [101 to 127 microns] long, .0003 to .0004 inches [7.6 to 10.1 microns] broad; spores filiform, nearly straight, .002 to .003 inches [50 to 76 microns] long; paraphyses filiform, numerous. Dead branches of leather leaf, *Cassandra calyculata*. Karner, N. Y. Aug.

Dr. H. D. House, State Botanist of New York, has kindly compared our specimens with Peck's type and regards them as the same.

We have found a few minor variations from Peck's description of the apothecial form. On the cranberry stem, as on Cassandra, the apothecia generally vary between 0.5 and 0.7 mm. in diameter, but on cranberry fruits they grow larger, in fresh specimens ranging from 0.5 mm. to 1.8 mm. with the majority 1 mm. or more in diameter. In our material, asci showed greater variation in length, measuring 58.8–117 by 5.0–7.7 microns. Ascospores in our specimens on Cassandra measured 55–74 by 2 microns, and on cranberry 39–74 by 1.5–3.8 microns, mostly 55–70 by 2–3 microns. Peck evidently considered the elongate, hyaline ascospores to be nonseptate, but we found that in mature condition they became clearly 4- to 7-septate, mostly 6- and 7-septate. Ascospore septation is also evident in stained sections of mature apothecia. Paraphyses often branch towards the apex. Figures 3 to 6, respectively, show the macroscopic appearance of apothecia on decayed cranberry, section of an apothecium on cranberry stem, a highly magnified portion of the hymenial layer, and mature and germinating ascospores. The end-rot fungus seems to be congeneric with the type of Godronia, *G. urceolus,* and should hereafter retain the name, *Godronia cassandrae* Pk.

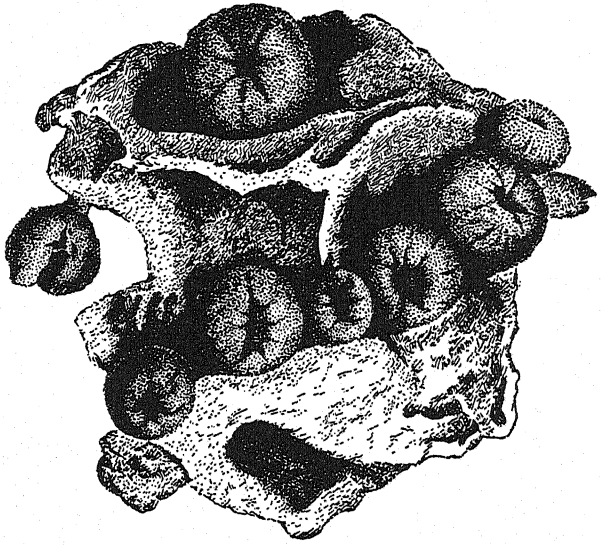


FIG. 3. *Godronia cassandrae*: Mature apothecia on mummied cranberry, X 15.

An examination of a specimen of *Dothiopsis myrtilli* Feltg. issued by F. Petrak in his Flor. Bohem. et Morav. Exs. No. 1609, as *Sirex cipulina myrtilli* (Oud.) Pet. shows that it is identical with *Fusicoccum putrefaciens* Shear. Petrak's specimens were found on *Vaccinium myrtilli* in Bohemia,

Apr. 30, 1923. Petrak (3), discusses this fungus and suggests that it may be the pycnidial form of *Godronia ericae* (Fr.) Rehm. This Godronia, however, is distinct from *G. cassandrae* Pk.

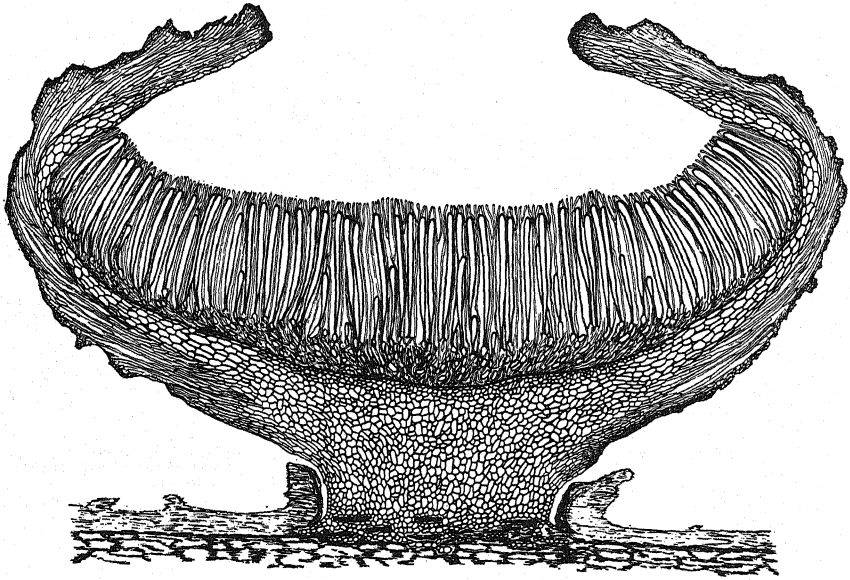


FIG. 4. *Godronia cassandrae*: Median section of apothecium cut longitudinal to cranberry stem, showing detailed structure of apothecium, X 137.

The apothetical form of *Godronia cassandrae* has been collected on stems and fruit of the cranberry in Massachusetts, Oregon, and Washington. Rudolph found one specimen on a fallen leaf in Washington. It has been found most frequently in the Pacific Coast region, where the climatic conditions seem to be more favorable for its development. As before stated, it has been collected also on Cassandra in New York and New Jersey.

Both pycnidia and ascomata of this fungus were collected also on dead twigs of *Vaccinum caespitosum* Mx. above timber line on Mt. Washington, N. H., July 12, 1922 (Shear and Stevens, Nos. 5159 and 5152).

A detailed description of the pycnidial form of the fungus *Fusicoccum putrefaciens* is not necessary here, as it has been described in detail elsewhere (5). Drawings of the pycnidia and pycnospores are reproduced in figures 1 and 2. The pycnidial form has been found on fruit and leaves of the cranberry, *Vaccinium macrocarpon* Ait., in New Jersey, Massachusetts, Michigan, Wisconsin, and Washington (5). In addition to collections on cranberry in the States named, Fusicoccum has been isolated from decaying berry tissue from the following hosts and localities: *V. macrocarpon*,

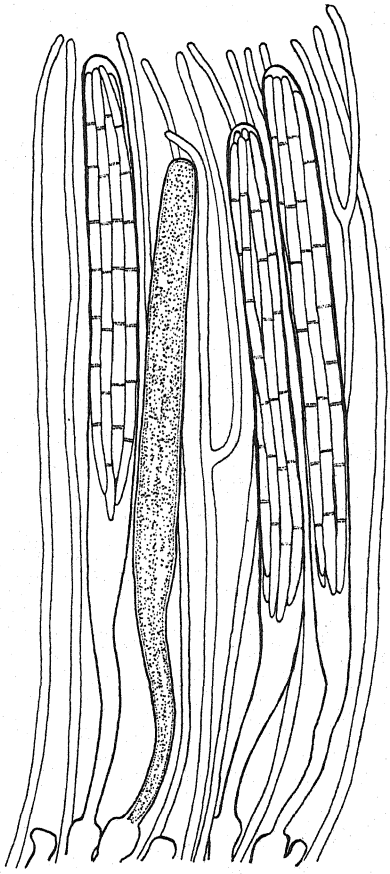


FIG. 5. *Godronia cassandrae*: Camera-lucida drawing of asci and paraphyses from section of apothecium on cranberry fruit, X 1000.

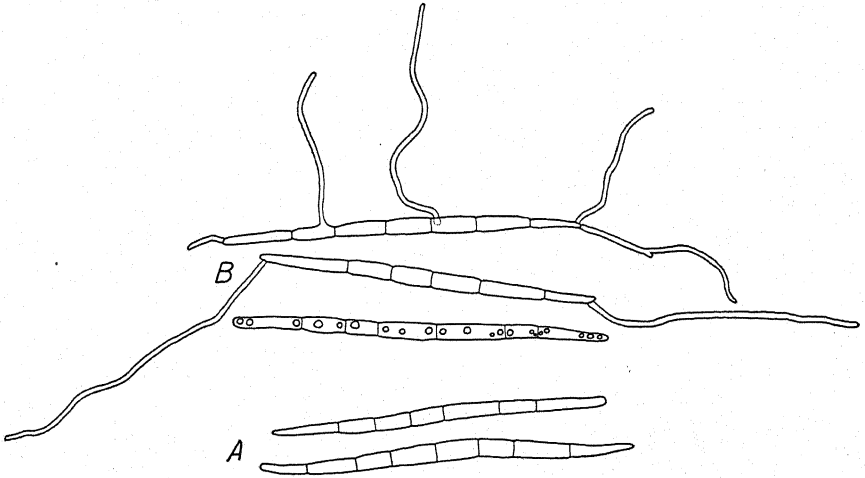


FIG. 6. *Godronia cassandrae:* A, mature, B, germinating ascospores, from apothecium

North Carolina and Maine (8), *V. Vitis-Ideae,* Maine (8) and Alaska, and *V. oxycoccus,* Alaska (8).

ECONOMIC IMPORTANCE

Investigations carried on for the past ten years by Stevens, Rudolph, Franklin, and the present writers (1, 4, 6, 8, 9, 10) have shown that end rot causes greater loss to cranberries during storage, transportation, and distribution than any other factor.

THE PROBLEM OF BERRY INFECTION

End rot of cranberries is distinctly a storage trouble, as the rot rarely develops before berries are harvested. Unfortunately, the exact time and mode of infection of the fruit have not yet been definitely determined. Various difficulties involved in carrying out conclusive inoculation experiments with the fungus have not been overcome. Stevens (8) has shown in experiments carried on in Massachusetts that the end-rot fungus was present in young berries at the time the flowers were dropping. He isolated the end-rot fungus from such berries as early as July 6. Bain (1) also obtained the fungus from very young cranberries in Oregon on July 10, 11, and 14, in successive years. In most cases the fungus was found to be in the blossom end of the young berry. Stevens and Bain have also cultured the fungus from surface-sterilized living stems and leaves of cranberry in Massachusetts and Wisconsin, respectively. Isolations made from fruit later in the growing season do not show a much greater percentage of berries infected than at the early stage of the fruit. This would seem to show that infection either occurs during the blossoming period or immediately after, or is possibly systemic.

The fruiting forms of the end-rot fungus are relatively scarce, especially in Massachusetts, and it is difficult to account for the abundant infection of berries over large areas. The spores of the pycnidial form, Fusicoccum, are ejected and held together in glutinous masses not adapted to wind distribution. The ascospores may be disseminated in part by wind, but these are so rarely found in eastern cranberry bogs as to appear inadequate to produce the infection which usually occurs. Infection of the blossom and of the fruit may occur through the agency of insect pollinators of the cranberry blossom or may result from the flood waters which are frequently used in spring to protect the young growth from frost injury. It seems possible that there may be some systemic infection of the vines which may enter the fruit through its pedicel, as numerous cases of stem-end rot as well as blossom-end rot occur. Yet, the fact that considerable reduction of end rot is obtained by spraying with Bordeaux mixture could scarcely be explained except on the supposition that infection

for the greater part takes place through the blossom or surface of the fruit. Further investigation of these points of fundamental importance will be undertaken.

DEVELOPMENT OF END ROT IN THE BERRY

There is strong evidence that the end-rot organism develops rapidly only in berries which have been weakened by mechanical injury, partial smothering, senescence, or some other cause. The rot is extremely rare in berries before maturity and during the early period of storage, but, as has been repeatedly shown (4, 9, 10), it steadily increases as berries approach senescence. Shear, Stevens, Wilcox, and Rudolph (6) have demonstrated that injuries brought about by careless handling of berries during harvesting, screening, and packing operations lead to excessive development of the rot. As long as berries are provided with satisfactory storage conditions and are not too old, end rot develops but slowly; but, following mechanical injury or aging of the fruit, Godronia soon becomes the predominating cause of spoilage.

TEMPERATURE RELATIONS

Stevens, in 1917, presented data showing that *Fusicoccum putrefaciens* (*Godronia cassandrae*) in culture is able to grow slowly at a temperature of 0° C. (7). He pointed out at that time that this relation was favorable to the development of the fungus in the colder cranberry regions of the country. In a series of unpublished cold-storage experiments conducted in the fall of 1928, Stevens and Bain repeatedly isolated Godronia from berries which rotted at an accurately-controlled temperature of 0° C., thereby proving that the fungus is capable of destroying berries at that temperature. The ability of the fungus to grow at low temperatures is a factor of great importance in storing and marketing cranberries, since late berries are generally held at temperatures too low for the development of most other cranberry rots.

CONTROL METHODS

Two distinct methods of reducing loss from end rot are available. The first and most effective is by spraying. Numerous experiments in different parts of the country have shown that spraying with Bordeaux mixture two or three times during the summer gives satisfactory control of this as well as other storage rots (1, 5). The most important applications of spray are those made immediately preceding and following the blossoming period. The second method of reducing end rot involves regulation of storage and handling processes to check or delay the development of rot in infected berries. Factors known (6) to have an important bearing in this connection include (a) thorough drying of berries before storage; (b) adequate

ventilation; (c) prevention of unnecessary bruising of fruit; and (d) storage at low temperature, which, while not so effective as against other storage fungi having higher minimum-temperature requirements than *Godronia cassandrae*, is nevertheless of very definite value.[1]

SUMMARY

Evidence is presented demonstrating genetic relationship between *Fusicoccum putrefaciens* Shear, the cause of cranberry end rot, and *Godronia cassandrae* Peck, originally described on *Cassandra calyculata*.

The present known distribution and hosts of the fungus are given.

The pathology of cranberry end rot is briefly discussed, and control methods are outlined.

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE.

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[1] Since this paper was written, the junior author has made single ascospore cultures from *Godronia cassandrae* and obtained typical pycnidia of *Fusicoccum putrefaciens*, as found on cranberry, thus completing the evidence of the identity of the fungi on the two hosts.

AIRPLANE PHOTOGRAPHY IN THE STUDY OF COTTON ROOT ROT[1]

J. J. Taubenhaus, Walter N. Ezekiel, and C. B. Neblette

INTRODUCTION

Stakman and his associates used the airplane in their studies[2] on the spread of cereal-rust spores through the upper-air currents. They caught spores of several genera of pathogenic fungi, conidiophores, pollen grains, glumes of grasses, and small insects on microscope slides exposed at elevations varying from 1,000 to 16,500 feet. Airplanes are used extensively in dusting for various insects and have been tried for plant diseases. So far as the writers are aware, however, no previous studies of plant diseases have been made by airplane photographs.[3]

DIFFICULTY IN PHOTOGRAPHING ROOT ROT

Cotton root rot, caused by the fungus *Phymatotrichum omnivorum* (Shear) Duggar, is one of the most serious plant diseases of the Southwest and entails annual losses running into millions of dollars. The disease causes definite dead areas or spots in the fields. These spots stand out prominently and are conspicuous even from considerable distances, as when viewed from moving trains. Yet, it always has been difficult or impossible to obtain satisfactory photographs showing the relative severity of root rot in naturally-infected fields or in experimental plots.

No matter how few or how many plants are killed by root rot in a given field, there is no general mass effect visible unless nearly 100 per cent of the plants are killed. In a field with 50 per cent loss from root rot, the remaining 50 per cent of the plants are as large as they would be were all the plants sound. Similarly, photographs of plants that have died from root rot in fields where nearly every plant has been killed would apply just as well to the occasional dead plants in fields where losses do not exceed 2 per cent.

With photography from the ground, the inevitable foreshortening in the picture emphasizes the few plants in the foreground and the comparative value of the photograph is based on these plants. Photographs are some-

[1] Published with the approval of the Director as contribution No. 462 of the Texas Agricultural Experiment Station.

[2] Stakman, E. C., Henry, A. W., Curran, G. C., and Christopher, W. N. Spores in the upper air. Journ. Agric. Research, 24: 599–606. 1923.

[3] This work has been mentioned already as follows: Neblette, C. B. Aerial photography for study of plant diseases. Photo-Era Mag. 58: 346. 1927. Airplane photography for plant disease surveys. Photo-Era Mag. 59: 175. 1928.

times obtained in which a few surviving, normal plants in the foreground obscure the great mass of dead plants even in fields in which almost every plant has been killed. Again, where root rot is scarce, it may either be overlooked in the photograph, or the picture may give the impression that the disease has involved most of the plants in the field. For comparative purposes, photographs must therefore be general views which include entire plots or fields and at the same time differentiate plants killed by root rot from the normal plants. This has been done with reasonable satisfaction by ordinary photography only for experimental plots of single rows.

It may be noted that this optical obscurity of losses from root rot is a hindrance also to rapid visual estimation of losses. When root rot occurs in more than one spot in a field estimates of losses are nearly always too low since the surviving plants are so much more conspicuous to the eye than those plants that have wilted.

On numerous occasions army fliers stopping over at College Station reported to us that root rot spots were readily visible from the air, even when flying at high altitudes. This suggested that root rot spots might perhaps be photographed more successfully from an airplane. One of us (Neblette) has made such photographs. The method appears so valuable as to suggest its use in furnishing estimates of losses as well as in furnishing better pictures of the results of field experiments with root rot.

AERIAL PHOTOGRAPHIC METHODS

Several test flights were made in October, 1927, near Substation No. 5 at Temple, where root rot is widespread. Some of the photographs secured at that time are shown in figure 1. Subsequent flights were made from Temple to Waco, a distance of seventy miles. It was found that the best times of day to make these exposures were at 10 A. M. and 3 P. M., or at other times when the sun was shining brightly. Results were not so good when exposures were made during cloudy weather or at noon because heavy shadows cast by the leaves of normal plants help to differentiate them from plants with root rot. The height varied from 250 to 500 feet. An ordinary Graflex camera, size 5 x 7, fitted with a Bausch & Lomb f 4.5 Tessar lens of 10-inch focus, was used with Eastman commercial panchromatic film and a K1 (light yellow) filter. An aero filter made especially for aerial photography probably would have given sufficient color correction to eliminate the usual slight atmospheric haze with less increase of exposure.

Ordinary hand or Graflex cameras are not used customarily in aerial photography, so it may be of interest to give the methods. It must, of course, be remembered that the camera is being used for purposes for which it was not designed and one must be prepared to accept larger percentages of failures, but comparatively good pictures may be secured by proper

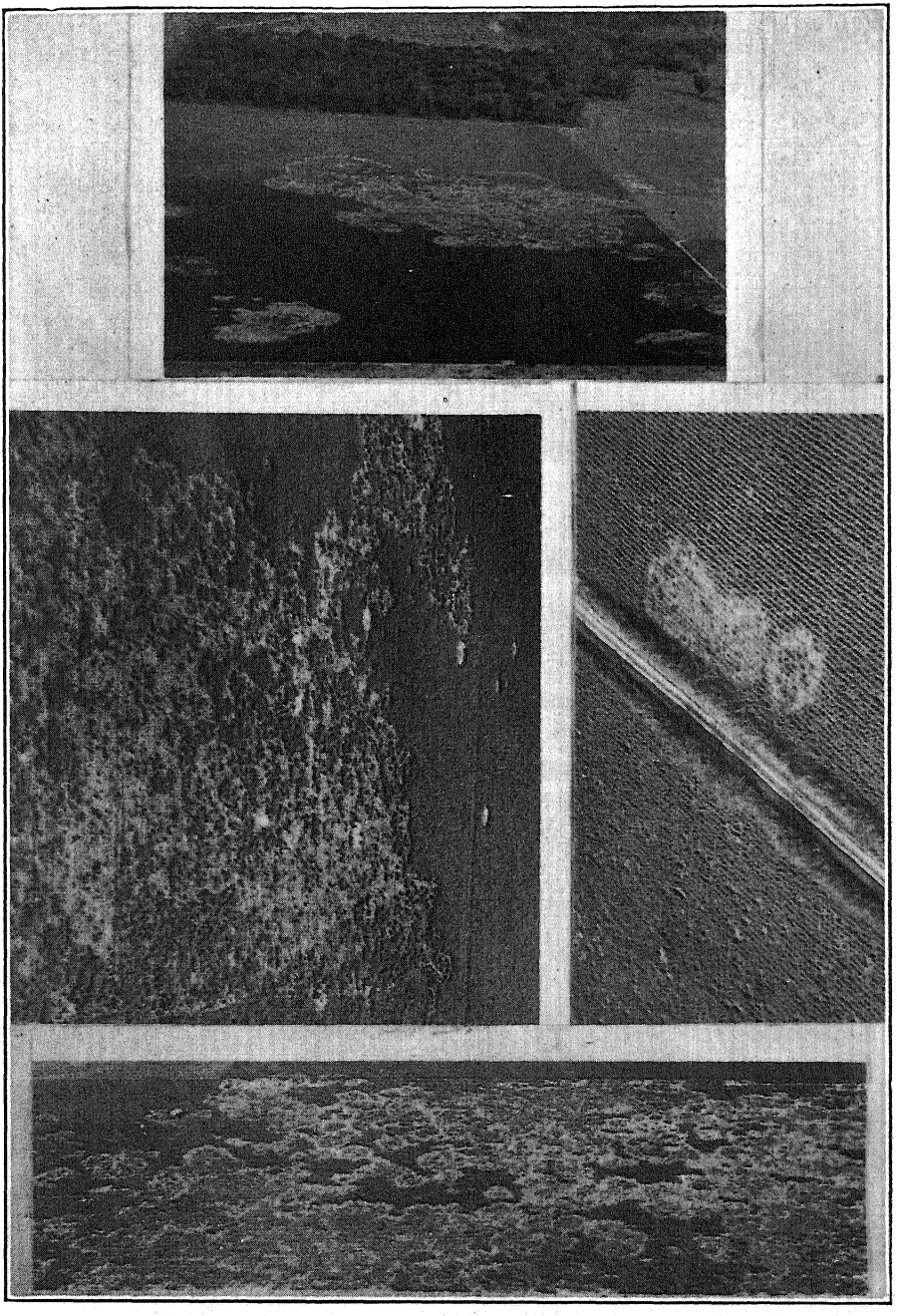


FIG. 1. Airplane views of four cotton fields showing root rot spots of various sizes and shapes. The dark regions in the photographs are areas of normal plants, and the light regions are the dead spots.

manipulation. As it is difficult to focus the camera while in the plane, the focus should be accurately adjusted to infinity before one leaves the ground and the lens secured in such fashion that the focus cannot be disturbed. The hood provided for focusing must be closed down as it would otherwise be torn off by the wind when the camera is held out over the side of the plane. A small direct vision finder attached to the side of the camera may be used to determine the area included in the picture.

Ordinary film may serve in clear atmospheric conditions and at low altitudes up to 500 feet. However, since panchromatic films may be obtained now not only in professional sized flat films, but also in rolls and packs of the usual sizes, there is no necessity for using any other type of film regardless of the requirements of the camera one is using. With panchromatic film an Aero 1 or a Wratten K1 filter is sufficient to give excellent contrast under usual conditions.

The elevation at which pictures are to be made depends on the purpose of the work, the focal length of the lens and the permissible exposure. The area covered in a single photograph will depend on the altitude at time of exposure, the focal length of the lens, and the angle of view in the case of oblique views. Table 1 gives the areas included in photographs at different altitudes.

TABLE 1.—*Dimensions of field included in vertical photographs with lenses of 6, 8, and 10 inches equivalent focal lengths at various altitudes*[4]

Altitude, feet	Dimensions of field included in photographs, feet		
	6 inch lens with 4 x 5 plate	8 inch lens with 5 x 7 plate	10 inch lens with 7 1/16 x 9 1/2 (18 x 24 cm.) plate
250	60 x 71	100 x 122	175 x 225
500	120 x 142	200 x 244	350 x 450
750	180 x 213	300 x 376	525 x 675
1000	240 x 284	400 x 488	700 x 900
1250	300 x 355	500 x 610	875 x 1125
1500	360 x 426	600 x 732	1050 x 1350
2000	480 x 568	800 x 976	1400 x 1800

While fairly sharp results may be obtained in aerial photography with a Graflex camera or with a focal-plane hand camera of the type represented by the Cycle Graphic, more accurate work would require a camera especially

[4] This table has been calculated for use with hand cameras and reflex cameras, such as the Graflex, at lower elevations than is customary with standard aerial cameras. It is not advisable to attempt to work with ordinary cameras from the high altitudes commonly used in survey work with aerial cameras.

designed for aerial photography. A comparatively wide range of such cameras is now available. Fully-recording mapping cameras such as the Fairchild require special installation in the plane and the price is prohibitive for all but those commercially engaged in aerial photography. The Folmer type K-5 camera is less expensive, but the other objection mentioned still holds. Both the Fairchild and the Folmer cameras use Eastman aerial film which is supplied in rolls 25 cm. (9½ inches) wide for 37, 70, or 100 exposures, 18 x 24 cm. An excellent camera for general airplane photography at a more moderate cost is the Folmer type A-1, making pictures 4 x 5 inches in size. This type of camera may be used for both oblique and vertical pictures but is not suitable for exact mapping purposes. It is possible to build a satisfactory camera of this type for considerably less cost than the commercial article, purchasing the lens and a suitable focal-plane shutter. Such a 5 x 7 camera built to the specifications of one of the writers is to be tested during the coming summer and fall.

SUMMARY

(1) Damage caused by cotton root rot is such as to make it difficult or impossible to secure from the ground photographs that show satisfactorily relative losses in fields or in plots of more than a single row.

(2) Airplane photographs have solved the problem by furnishing views which include entire fields and at the same time differentiate plants killed by root rot from the normal plants.

(3) Suggestions are given on airplane photography with ordinary hand cameras.

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A NEW HOST OF SUGAR BEET CURLY TOP

RUTH COLVIN STARRETT

INTRODUCTION

In a publication by Carsner[1] the following families have been reported to be susceptible to curly top: Amaranthaceae, Caryophyllaceae, Chenopodiaceae, Geraniaceae, Leguminosae, Malvaceae, Polygonaceae and Urticaceae. Severin and Henderson[2] have mentioned three families to be naturally infected with curly top, namely, Chenopodiaceae, Leguminosae and Cucurbitaceae.

Oxalis stricta L., according to the International Code of Classification belongs to the Geraniaceae family but the more recent edition of Gray, and also Britton and Brown, have assigned it to a new family, Oxalidaceae. The common occurrence of Oxalis and its very high acidity make it an interesting addition to the list of susceptible plants. The acidity for normal leaves and young stems is pH 2.45, but for the young leaves it is pH 2.23.[3]

GREENHOUSE EXPERIMENTS

In the investigations carried on at Arlington, Virginia, during the year 1928, experiments were made to determine the relationships of curly top in *Oxalis stricta* and sugar beets, and the ability of *Eutettix tenellus* (Baker) to transmit this disease from Oxalis to Oxalis, from Oxalis to beet, and from beet to Oxalis.

The first diseased plant of *Oxalis stricta* appeared as a volunteer growing in a pot along with a caged beet plant affected with curly top. This Oxalis plant had pronounced papillae arranged along the veins on the under side of the leaves varying in size and shape as in curly top of sugar beet. The entire plant was distorted in appearance due to the curled and twisted leaves and the reaction to infection was so severe that no normal leaves developed (Fig. 1, A). It remained green in color but considerably dwarfed, although the presence of the disease did not interfere with the regular seed production.

Three types of transmission experiments were tried, then each one repeated entirely with confirmatory results. One table for each type of experiment is given in this paper to demonstrate the results.

[1] Carsner, E. Susceptibility of various plants to curly-top of sugar beet. Phytopath. 9: 413–421. 1919.

[2] Severin, H. H. P., and C. F. Henderson. Some host plants of curly top. Hilgardia 3: 339–384. 1928.

[3] The writer is indebted to Dr. A. M. Hurd-Karrer for the pH determinations.

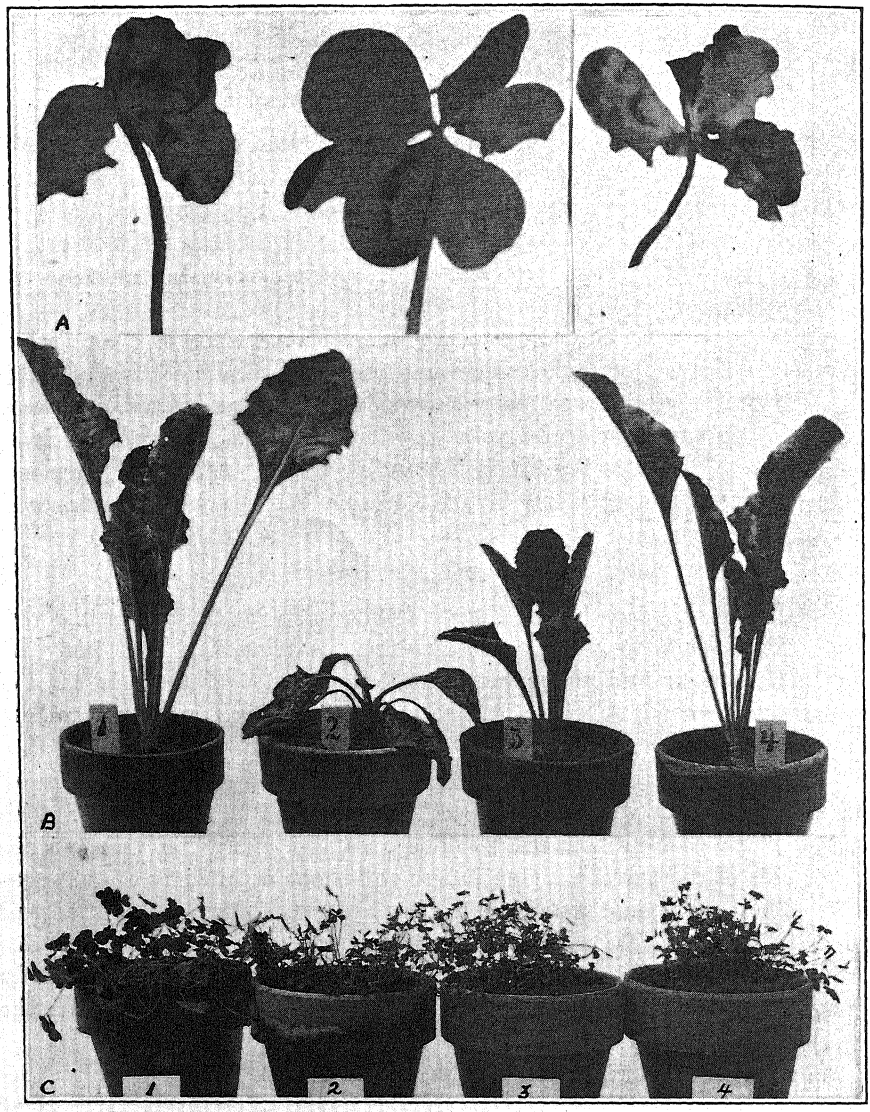


FIG. 1. Plants used in experiments 1 and 2. A. Typical effects of curly top upon the leaves of *Oxalis stricta*. B. Sugar-beet plants with typical curly-top symptoms produced by leaf hoppers previously fed upon the diseased Oxalis leaves, shown in A. C. Oxalis plants in which curly top was reproduced by leaf hoppers fed on the sugar-beet plants, shown in B.

In order to determine if the disease were curly top the plant was isolated in a large cage and nonviruliferous individuals of *Eutettix tenellus* were allowed to feed on it. After a week of uninterrupted feeding, these leaf hoppers were removed to small leaf cages by the suction-pipe method, two insects in each cage, and attached to healthy leaves of young sugar-beet seedlings. Two days were allowed for feeding on the beet plants and the leaf hoppers were then returned to the original Oxalis plant. Curly-top symptoms appeared in twenty-four days on the sugar beets and quickly became severe (Fig. 1, B, Table 1).

TABLE 1.—*Transmission of curly top of Oxalis to sugar beet by Eutettix tenellus*

Date of inoculation Nov. 6, 1928

Beet seedling no.	No. of insects	Results			
		11–30–28	12–7–28	12–10–28	1–11–29
I	2	Normal	Normal	Normal	Normal
II	2	Smallest leaves veinlets cleared	More decided clearing	Definite case of curly top	Positive case
III	2	?	Decided veinlet clearing	Positive curly top	Positive case
IV	2	Normal	Normal	Normal	Normal
	Checks				
I	2	Normal	Normal	Normal	Normal
II	2	Normal	Normal	Normal	Normal

In order to complete a cycle of transmission, nonviruliferous leaf hoppers were caged on one of the beet seedlings that had developed a typical case of curly top (Table 1) and, after the regular time allowed for feeding, were removed to four healthy Oxalis plants. Three of these plants developed unmistakable signs of the disease in about one month (Fig. 1, C) (Table 2).

Leaf hoppers were then placed on one of the curly-top Oxalis plants and later transferred to four healthy Oxalis plants for two days, four viruliferous leaf hoppers in each large cage. Two of these plants showed definite signs of curly top in about eighteen days (Table 3).

Because of the reported transmission of a disease of sugar beet resembling curly top in Argentina by leaf hoppers of the genus Agallia, two species that had been found to feed on beets at Arlington, *A. constricta* and *A. sanguinolenta,* were also used in these experiments.

All the conditions were duplicated, the periods of feeding being exactly parallel with those used for *Eutettix tenellus,* greenhouse conditions were

TABLE 2.—*Transmission of curly top of beet to Oxalis by Eutettix tenellus*

Oxalis stricta no.	No. of insects	Results			
		1–7–29	1–12–29	1–18–29	1–21–29
		Date of inoculation Dec. 10, 1928			
I	4	Small leaf veinlets cleared	Definite curly top	Curly top	Curly top
II	4	Normal	Normal	Normal	Normal
III	4	One leaf with papillae	Definite case	Curly top	Curly top
IV	4	Decided papillae	Definite case	Curly top	Curly top
	Checks				
I	4	Normal	Normal	Normal	Normal
II	3	Normal	Normal	Normal	Normal

the same, and both Oxalis and beets were used as host plants, but in each experiment the results were negative.

TABLE 3.—*Transmission of curly top from Oxalis to Oxalis by Eutettix tenellus*

Oxalis stricta no.	No. of insects	Results		
		1–26–29	2–2–29	2–5–29
		Date of inoculation Jan. 15, 1929		
I	4	Young leaf slightly curled	Positive curly top	Positive curly top
II	4	Normal	Normal	Normal
III	4	Suspicious	More definite markings	Positive curly top
IV	4	Normal	Normal	Normal
	Checks			
I	7	Normal	Normal	Normal
II	10	Normal	Normal	Normal

SUMMARY

The results of the experiments prove that curly top can be carried from *Oxalis stricta* to beet and inversely through the agency of *Eutettix tenellus*.

Curly top, in Oxalis, checks the growth of the plant but never results in a total destruction, even in cases of severe infection.

In Oxalis, curly top usually is manifest in a conspicuous development of protuberances or papillae on the under surface of the leaves.

Two species of Agallia, *A. constricta* and *A. sanguinolenta*, were tested as vectors with negative results.

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STORAGE ROTS OF CRANBERRIES IN THE 1928 CROP

NEIL E. STEVENS AND HENRY F. BAIN

During each of the past three years the writers have attempted to measure quantitatively the actual storage rot caused by various fungi in cranberries in commercial storage. The chief interest attaches, of course, to a comparison of the results obtained in different years, from different regions, and with different varieties. Where there are so many variable factors, the results cannot be made strictly comparable, yet they do permit of some comparison and in many ways are more interesting than if artificial conditions, such as constant storage temperatures, had been maintained. The most serious break in our records for this year is that, largely because of the very early harvest in that region, no Oregon or Washington berries were included in the storage test.

METHODS

The methods followed were substantially those of 1926 and 1927,[1,2] fully described in earlier papers. The chief difference, other than the omission of berries from the West Coast, was that the berries had to be stored in the third story of the building in Chicago; whereas, in 1926 and 1927, they had been kept in the basement. As the windows of this storeroom were open, the berries were subjected to much lower temperatures in October, 1928, than in the same month in 1926 or 1927 (Table 1). On the other hand, the November temperatures of 1928 corresponded very closely to those of 1926, and the December temperatures for 1928 were well above either 1926 or 1927.

TABLE 1.—*Approximate Storage Temperature, Chicago, in Degrees F.*

	1926	1927	1928
October	65–55	70–60	62–46
November	53–43	58–48	55–44
December	40–30	48–35	50–40

RESULTS

The results are shown graphically in figure 1. Comparing the keeping quality of the stored lots for the three years, it is evident that, for Wisconsin, the keeping quality of the Howes was better in 1927 than in 1928 (no

[1] Stevens, N. E., and H. F. Bain. Storage Rots of Cranberries in the 1926 Crop. Phytopathology, 17: 649–665, 1927.

[2] ———, ———. Storage Rots of Cranberries in the 1927 Crop. Phytopathology, 18: 809–814, 1928.

Wisconsin Howes were included in the 1926 test), and for McFarlins, better in 1927 than in either 1926 or 1928. For Massachusetts, both varieties under test showed the best keeping quality in 1926 and the poorest in 1927, while for New Jersey, both varieties were poorest in 1927 and best in 1928.

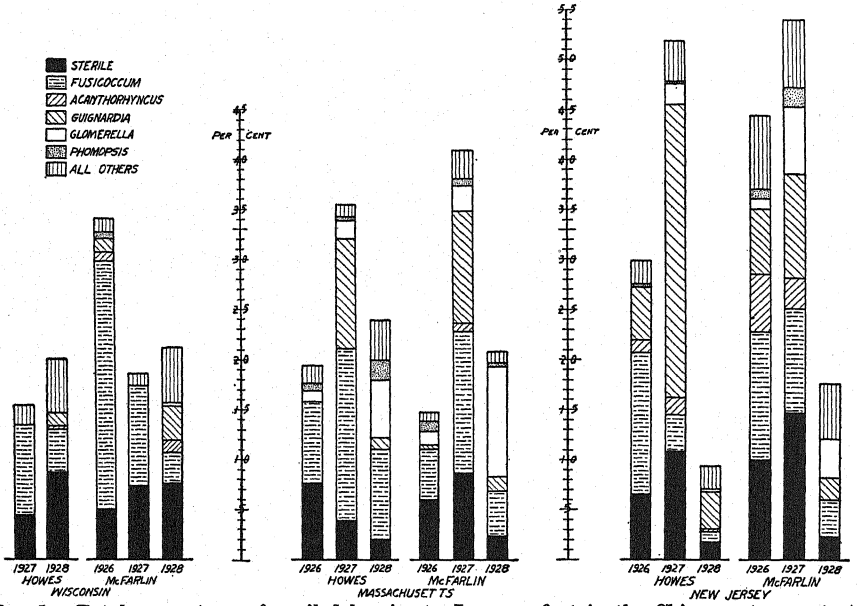


FIG. 1. Total percentage of spoiled berries to January first in the Chicago storage tests for the crops of 1926, 1927, and 1928, with proportions initiated by different fungi and sterile breakdown shown by suitable conventions.

It can not be assumed that the berries used in these storage tests are always representative of the crops of the various regions. For example, the Massachusetts Howes used in 1928 were somewhat too green when harvested to show the best keeping quality or to fairly represent the Massachusetts crop of that year. In some cases, however, the results shown in figure 1 seem to agree very well with what is known of the keeping quality of the entire crop of their respective regions. The results for New Jersey, for example, show a surprising agreement with a keeping quality index worked out by a wholly different method, that is, the comparison of the market claims allowed for decay in 1926, 1927, and 1928.[3] All observations agree in showing that the keeping quality of the crop of the different regions varies independently.

[3] Stevens, N. E. Six Years' Experience in Forecasting the Keeping Quality of the Cranberry Crop in Wareham and Carver, Massachusetts. Proceedings American Cranberry Growers' Association, 59: 8–16, 1929.

COMPARATIVE KEEPING QUALITY OF THE HOWES AND MCFARLIN VARIETIES

Special importance is attached at this time to a comparison of the keeping quality of the Howes and McFarlin varieties. The Howes has long been recognized as the standard late variety of cranberry. The McFarlin is the most important of the so-called fancy or large size cranberries. It has come into prominence during the past few years because of its very high degree of resistance to the destructive false-blossom disease.

A careful examination of figure 1 shows that, for any given region and year, the keeping quality of the McFarlin up to January first is only slightly inferior to that of Howes. In Massachusetts, indeed, McFarlins were better both in 1926 and 1928 than the Howes. The superior keeping quality of the Howes variety is shown more markedly during January and February, but in many years a very large proportion of the crop is marketed before January.

Regarding the importance of the different fungi, the only outstanding variation from earlier years is the large percentage of Glomerella in both Howes and McFarlin in the Massachusetts crop. It may be noted that the amount of sterile breakdown is very low in both varieties for Massachusetts and New Jersey in 1928.

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STEM RUST OF WHEAT IN PERU[1]

E. V. Abbott

Stem rust is the most important disease of wheat in Peru. It occurs every year in epidemic form in many of the highland valleys of the mountains where this crop is grown extensively and, on the coast, it is so destructive that wheat is no longer cultivated there. It is doubtful whether wheat could compete with either cotton or sugar cane as a major crop on the coast, even if a rust-resistant variety were available, but it could supplement them and find large use as a food for livestock if not for human consumption. The same is true of barley, the cultivation of which is at present limited to the mountain valleys because of rust.

In an effort to obtain a rust-resistant wheat for the coast as well as the highlands, sixty varieties were imported for field study at the Lima experiment station during 1928, the seed of which was furnished through the courtesy of the United States Department of Agriculture and several of the State experiment stations in the United States. Both common and durum wheats were included, as well as two emmers.

The first planting was made in rows one meter apart, the middle of June, which, in Peru, is the beginning of the winter season. A second planting of several of the varieties was made in August. Inasmuch as rain does not fall on the Peruvian coast, water was supplied by irrigation. During the period from June to October heavy fogs and mists are very common at Lima, but they do not furnish sufficient moisture for crop growth.

The results obtained with eleven of the imported varieties, which are representative of the sixty tested, and one native wheat, are briefly summarized in table on the following page.

It will be noted that Pusa-4 showed the lowest percentage of infection among the common wheats and that it matured grain. This was due entirely to its earliness and consequent rust escape rather than to rust resistance. The same variety planted in August rusted so heavily that not a single kernel matured, which was also true of the other common and the durum varieties.

The outstanding feature of the results and the point of most importance are the high degree of infection of Khapli emmer. In the June planting of this variety, infection by stem rust was 50 per cent, with the degree of infection of individual plants heavy, while in the August planting 100 per cent of the plants rusted, the degree of infection being classed as very heavy. The heads produced from the late sowing were smaller than normal and the grain was slightly shrivelled.

[1] Contribution from the Experiment Station of the National Agrarian Society, Lima, Peru. Published with the approval of the Director.

TABLE 1.—*Infection of wheat varieties by Puccinia graminis tritici (Pers.) Eriks. and Henn.*

Variety	Per cent plants rusted[1]	Degree of infection[2]	Grain matured
1. Little Club	100	Very heavy	No
2. Hope	100	Heavy	Yes; grain slightly shrivelled
3. Kanred	100	Heavy	Yes; grain shrivelled
4. Marquis	100	Very heavy	No
5. Monad	100	Very heavy	No
6. Pentad	100	Very heavy	No
7. Pusa-4	88	Medium	Yes; grain normal
8. Webster	100	Very heavy	No
9. White Federation	100	Very heavy	Few plants matured
10. Native common wheat	100	Very heavy	No
11. Khapli emmer	50	Heavy	Yes; grain normal
12. Yaroslav emmer	93	Heavy	Yes; grain slightly shrivelled

[1] Based on counts of 500 to 1000 plants.

[2] Infection of individual plants, based on number and size of pustules.

The high degree of infection of Khapli indicates the presence of a new physiologic form of *Puccinia graminis tritici* in Peru. According to the key of Stakman and Levine[2] for the identification of physiologic forms of this fungus, the Peruvian form would be form XV; but in view of the great difference in the percentage of infection Khapli in Peru and the maximum previously reported,[3] it seems probable that they are not identical. Infection experiments employing the twelve differential hosts of Stakman and Levine have not yet been completed. Studies are being made to determine whether there are morphologic differences between the Peruvian form and form XV.

In considering the problem of stem rust epidemics in Peru the questions naturally arise as to how the causal fungus survives from one crop season to the next, and what rôle species of Berberis may play in dissemination of the disease. As opportunities have arisen from time to time, observations have been made in the wheat-growing valleys of the mountains of Peru with these questions in mind.

Species of Berberis are common in the Peruvian Andes, seven members of the genus being found there, according to Weberbauer.[4] The writer has

[2] Stakman, E. C., and M. N. Levine. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bul. 8. 1922.

[3] Clark, J. A., J. H. Martin, and E. C. Stakman. Relative susceptibility of spring-wheat varieties to stem rust. U. S. Dept. Agr. Dept. Circ. 365. 1926.

[4] Weberbauer, A. Die Vegetation der Erde. XII, Die Pflanzenwelt der peruanischen Anden. Leipzig, 1911.

observed two species of barberry growing along the roadsides bordering fields of rusted wheat and on the surrounding hillsides in great abundance near Arequipa, Cuzco, Huancayo, Jauja, and Tarma, but in no instance were they infected with *Puccinia graminis*. The elevations of these valleys vary from nine to thirteen thousand feet above sea level. The observations thus far made are too limited, however, to permit the conclusion that the barberry plays no part in the life cycle of stem rust in Peru.

Another point not yet determined is whether the urediniospores of the fungus are able to survive from one crop season to the next in the same locality in which they are produced. It is possible that at high altitudes they do not survive the cold and that, on the coast, the high temperatures destroy their germinability. But, even though they be not killed by unfavorable environmental conditions in any given locality, or though there be no alternate host present on which they may pass the intervening period, there is such a succession of crops at the different altitudes in Peru that it seems probable the fungus might easily live continuously on wheat, merely passing from one district to another. Throughout the entire year there is not a lapse of more than about three months when wheat is not growing somewhere in the mountain valleys, the stage of development varying gradually with the differences in elevation. In view of what is known regarding the carrying of rust spores by air currents, it is not at all difficult to conceive of the fungus living continuously on wheat and being carried from one section to another by the wind. This appears to offer the only probable explanation of what occurred at Lima in 1927 when experimental sowings of wheat were made near Lima by the Federal Department of Agriculture. So far as is known, this was the first time in many years that wheat had been planted in this region. A heavy and general infection by *Puccinia graminis tritici* resulted, the disease appearing almost simultaneously in all parts of the field of about two hectares in size. There is a possibility that the fungus hibernates in this section on some wild grass, but no such host has yet been discovered. Wind-blown spores from the mountains seem to offer the most plausible explanation of these results, as well as those obtained at the Experiment Station in 1928.

DEPARTMENT OF PLANT PATHOLOGY,
ESTACION EXPERIMENTAL AGRICOLA DE LA
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LIMA, PERU.

RELATION OF TIME OF ROGUING TO THE SPREAD OF SPINDLE TUBER IN SEED POTATO PLATS[1]

H. O. WERNER

Early roguing of potato seed plats is commonly advised as a means of controlling virus diseases, since by removing the source of infection early in the season, there is supposedly less opportunity for disease to be transmitted to healthy plants. Some data upon the effect of early caging and early harvesting in relation to the spread of spindle tuber were presented in an earlier paper. It has also been shown that spindle tuber spreads more rapidly under irrigation than on dry land, and under eastern or central Nebraska early crop conditions than under the late crop conditions in the western dry-land areas, Werner.[2] Therefore, it seemed advisable to determine the extent to which spindle tuber can be controlled by the early roguing of seed plats planted early, under irrigation, at North Platte.

EXPERIMENTAL PROCEDURE

In April, 1925, spindle-tuber[3] Triumph potatoes were planted under irrigation at North Platte, Nebr., in every 5th hill in rows of certified Irish Cobbler potatoes, thus providing 20 per cent of known original infection. The Irish Cobbler seed had been grown on dry land in northeastern Wyoming and contained less than 1 per cent of spindle tuber. Triumph spindle-tuber potatoes were used because of the ease of distinguishing them from the Irish Cobbler, thus permitting the definite separation of the spindle tubers in the Irish Cobbler strain from the spindle-tuber Triumphs inserted as sources of infection. Schultz and Folsom[4] found that the spindle-tuber disease is not altered by transmission from one variety to another, and also that intervarietal transmission occurs as readily as intravarietal transmission. About 50 per cent of the plants had emerged by May 25. The Triumph spindle-tuber plants were removed from certain blocks (3 adjacent rows) at three-week intervals, beginning June 10.

The plats reserved as controls, in which no spindle tuber was planted, were hardly reliable control plats, since they were in the same general

[1] Published with the approval of the Director as paper No. 75 Journal series, Nebraska Agricultural Experiment Station.

[2] Werner, H. O. The spindle-tuber disease as a factor in seed potato production. Nebr. Agr. Exp. Sta. Res. Bul. 32. 1926.

[3] In this paper no effort is made to differentiate between spindle tuber and unmottled curly dwarf, because when the experiment was started the symptom differences were not clearly understood.

[4] Schultz, E. S., and Donald Folsom. Transmission, variation, and control of certain degeneration diseases of Irish potatoes. Jour. Agr. Res. 25: 43–117. 1923.

area, and the outside rows of these plats were adjacent to other experimental lots. A more satisfactory control was provided in a larger irrigated planting of this stock which was made in an isolated plat in a corn field.

The Irish Cobbler tubers produced in these plats were weighed according to grade. Special attention was paid to the prevalence of spindle tubers in each lot of potatoes. The spindle-tuber Triumphs produced in these plats were discarded at harvest time. Irish Cobbler seed potatoes were selected at random from the center row of each plat for planting in comparative trials under irrigation in 1926. In these trial plats there were duplicate plats of 3 rows each, the rows being 3 rods long.

At harvest time the tubers were carefully graded by size and all tubers of No. 1 size, showing definite spindle-tuber symptoms, were sorted out and weighed separately. This same procedure was duplicated in another experiment conducted during the years 1926 and 1927. The results for both experiments are reported and discussed together, because the results are fundamentally the same.

EXPERIMENTAL RESULTS

Seed plats. There was no evidence in either year of any current season spindle-tuber infection in the Irish Cobblers, due to the interplanting of spindle-tuber Triumphs. The plants and tubers were examined critically for any evidence of spindle tuber. The spindle-tuber readings reported in table 1 indicate that about the same percentage of spindle tuber was produced in the Irish Cobbler plats, into which no spindle-tuber Triumphs were planted, as in these plats from which the inserted Triumphs spindle-tuber plants were never removed. Undoubtedly the spindle-tuber potatoes sorted out were produced by plants grown from infected seed rather than as a result of current-season infection. There was much variation in yield from the different seed plats but none of this could be attributed to the inserted spindle tubers.

Comparative plats. When the tubers produced in the seed plats were tested in the comparative trial plats the following year it was found that when roguing of the seed plats had been delayed beyond June 10, there was a marked decline in yield and a great increase in the percentage of spindle tubers and small potatoes (Fig. 1 and Table 1).

The total yield from the various lots in the comparative plats decreased as the removal of the spindle-tuber plants had been delayed the previous year (Fig. 1, A). The differences in yield, due to roguing date, were more pronounced when only No.-1-size healthy tubers were considered.

Spindle tuber was responsible for the production of many small tubers. A positive correlation existed between the percentage of No.-1-size spindle tubers and the percentage of No. 2 and No. 3-size tubers.

TABLE 1.—*The effect of interplanting healthy Irish Cobbler seed potatoes with spindle-tuber Triumphs (rate of 4 to 1) upon yield and percentage of spindle tuber during the year seed tubers were produced, and the following year when grown in comparative plats. Seed plats and comparative tests planted under irrigation.*

A. Experiment conducted in 1925 and 1926

Treatment in 1925	Total yield, bushels per acre		Spindle tubers of No. 1 size—per cent of total yield	
	1925 (Seed plat)	1926 (Comparative plat)	1925 (Seed plat)	1926 (Comparative plat)
Check (grown in Wyoming on dry land)	*	346	*	1.2
No spindle-tuber Triumphs inserted in isolated plat	*	259	*	14.7
No spindle-tuber Triumphs in nonisolated plat	450	304	2.8	22.4
Triumph spindle-tuber plants removed June 10	390	264	0.5	26.3
Triumph spindle-tuber plants removed July 1	408	244	1.9	52.0
Triumph spindle-tuber plants removed July 20	414	202	1.2	63.5
Triumph spindle-tuber plants removed Aug. 10	550	177	1.1	61.4
Triumph spindle-tuber plants not removed	498	160	2.3	59.7

* Data not recorded in these seed plats.

B. Experiment conducted in 1926 and 1927

Treatment in 1926	1926 (Seed plat)	1927 (Comparative plat)	1926 (Seed plat)	1927 (Comparative plat)
Check (grown in Wyoming on dry land)	*	322	*	1.1
No spindle-tuber Triumphs inserted in isolated plat	*	307	*	1.1
No spindle-tuber Triumphs in nonisolated plat	346	209	1.5	4.6
Triumph spindle-tuber plants removed June 10	283	194	1.5	4.1
Triumph spindle-tuber plants removed July 10	303	88	2.0	32.4
Triumph spindle-tuber plants removed July 20	340	56	1.6	35.7
Triumph spindle-tuber plants removed Aug. 10	219	112	2.4	19.9
Triumph spindle-tuber plants not removed	269	127	1.6	8.7

* Data not recorded in these seed plats.

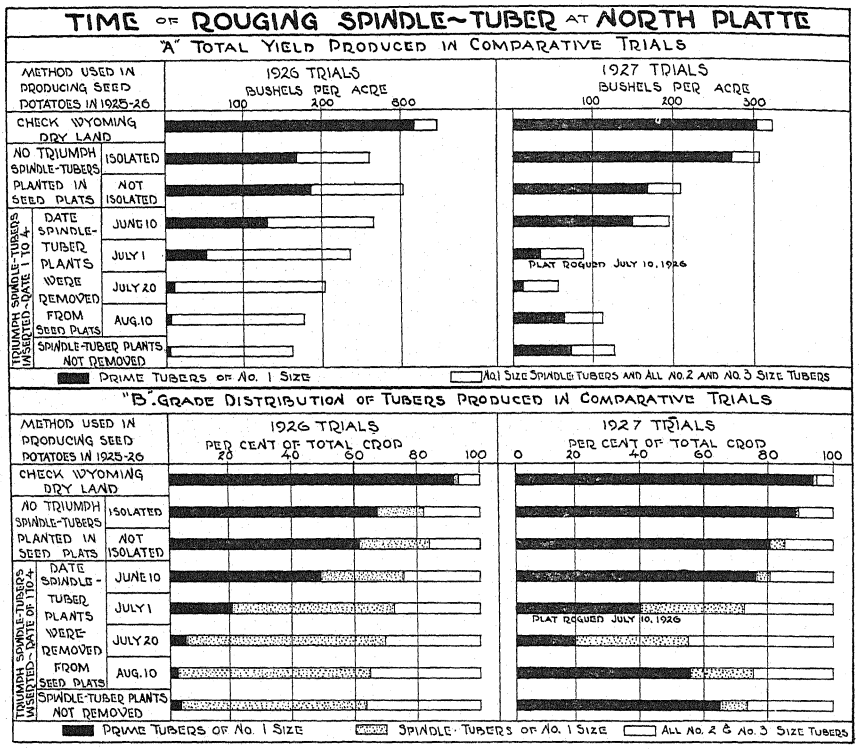


FIG. 1. The effect of the time of roguing interplanted spindle-tuber Triumphs from Irish Cobbler seed plats in relation to yield, grade, and spindle-tuber content the following season. Dates refer to years of comparative trials. Each year a new line of Irish Cobbler seed was planted in the seed plats.

In 1925 conditions were more favorable for transmission than in 1926. This accounts for the percentage of spindle tuber being greater in the 1926 than in the 1927 comparative plats, as shown by comparable lots. In 1926 the plants in the plats rogued July 10, July 20, and part of the August 10 plat remained green until harvest time in September; whereas, the plants in the other plats matured about the middle of August, due to accidental local soil-moisture shortage. The result of this difference in maturity was seen in the spindle-tuber content and yield in the 1927 comparative plat. The early maturing plats—even though not rogued or rogued late—produced seed with less spindle-tuber infection than did the early-rogued plats in which the plants remained green till the end of the season.

CONCLUSIONS

Spindle-tuber Triumphs, interplanted in seed plats among healthy Irish Cobblers (1 to 4), were rogued out at intervals of approximately three weeks.

Under the conditions of this experiment, *i.e.*, the production of seed potatoes as an early crop (April to September) under irrigation at North Platte, there were no current-season symptoms of spindle-tuber infection in either the plants or tubers produced in these seed plats; neither was the current-season yield appreciably affected. However, the comparative plantings the following year indicated that considerable infection had occurred as manifested by a high percentage of spindle tuber and reduced yields. Roguing done June 10 (7 weeks after planting) was reasonably effective but roguing after that date was of little value. In this experiment we knew the exact location of the diseased plants which were to be rogued. Such precise practice is not possible in commercial fields. Early maturity due to drought was quite effective in preventing the spindle-tuber virus from reaching the seed tubers.

The presence of a small amount of spindle tuber in Irish Cobblers did not always markedly diminish the total yield, but it did reduce the amount of primes by reducing the size of the tubers and by increasing the number of rough, bulging-eye tubers, thus detracting from their appearance and market value.

DEPARTMENT OF HORTICULTURE,
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COLEODICTYOSPORA, A NEW GENUS OF DEMATIACEAE

VERA K. CHARLES

In examining some diseased sugar-cane material from Cuba, collected by Dr. J. R. Weir, a fungus was observed which upon close study proved to be a new genus. Its morphology places it in *Dematiaceae* under Dictyosporae (Figs. 1 and 2). One conspicuous character of the fungus is a hyaline envelope which envelops the spore. This envelope may be limited to the spore proper or on occasions it may surround the stipe.

The amount of material for study was extremely limited and no inference can be drawn relative to any pathological significance.

COLEODICTYOSPORA

Hyphae scanty, simple or sparingly branched, short, procumbent or ascending, septate, at first hyaline, later colored; conidiophores fasciculate, erect, mostly simple, very occasionally branched, septate, flexuous, delicate, easily separated from the basal mycelium; conidia transversely oblong or inversely reniform, clathrate, clay color to deep olive, surrounded by a semi-gelatinous, hyaline diaphanous vescicle.

COLEODICTYOSPORA CUBENSIS

Hyphae scanty, procumbent to ascending, septate, hyaline to light fuscous; conidiophores densely fasciculate, slender, mostly simple, irregularly septate, 70–85 x 3.5–5 μ, hyaline, basal portion slightly colored in age, delicate, easily detached from the basal mycelium; conidia transversely oblong or inversely reniform, sometimes slightly constricted, constriction very occasionally under pressure total, separating the conidia into two equal parts, clathrate, tawny-olive, 42–50 x 20–22 μ, vescicle broadly ovate, diaphanous, later disappearing, sometimes enclosing the conidiophores, apical portion rugose-striate on drying, 60–55 x 40–45 μ.

On *Saccharum officinarum*

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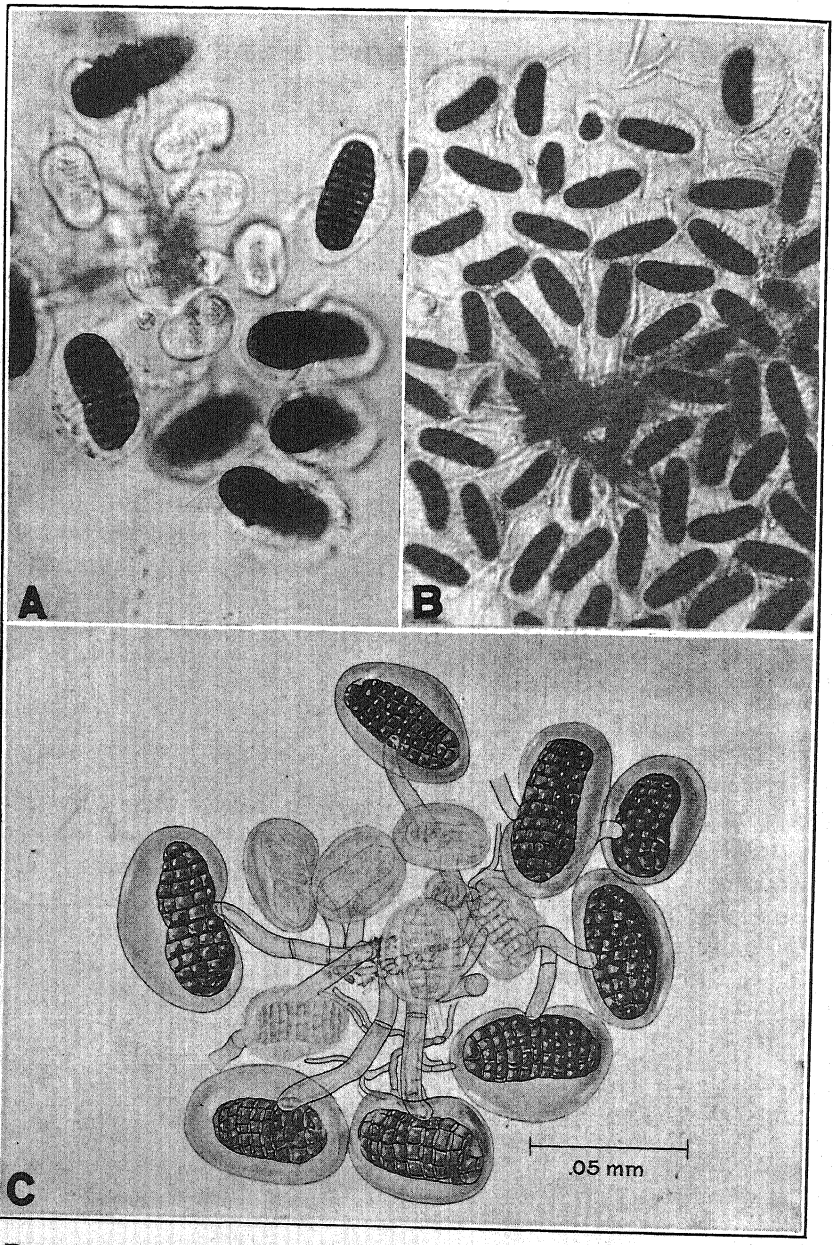


FIG. 1. A. Fascicle of conidiophores showing conidia in different stages of development. B. Fascicle of conidiophores with conidia. C. Interpretative drawing with aid of camera lucida and photograph of surface view of fascicle of conidiophores showing conidia in different stages of development.

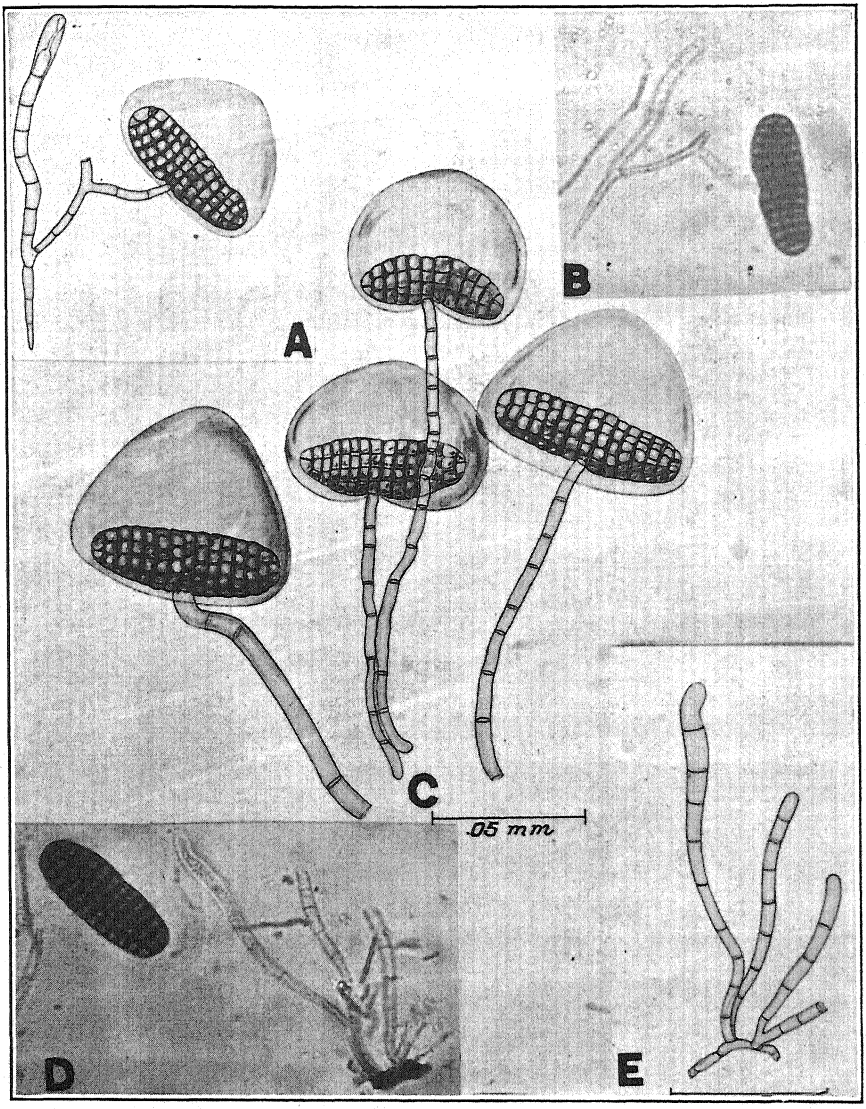


FIG. 2. A. Interpretative drawing with assistance of camera lucida and photograph of single spore showing branched conidiophore. B. Photograph of single spore showing branched conidiophore. C. Individual spores. Interpretative drawing with assistance of camera lucida and photographs. D. Group of conidiophores with conidia detached. E. Interpretative drawing of conidiophores.

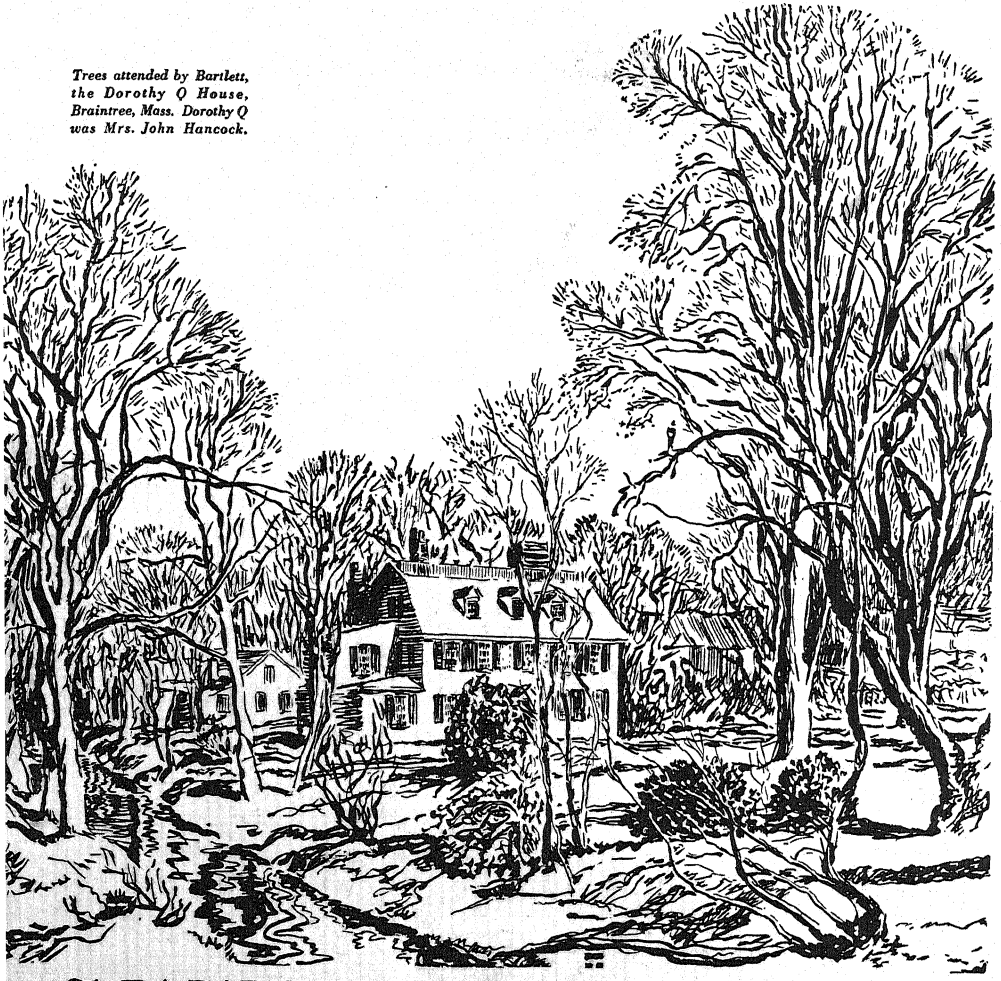
Here, too, it's safer to rely on science

PRICELESS heritages, these landmarks of our history. And how fitting that they should be preserved to posterity in their original form and setting. When their surroundings mean so much in maintaining their sentimental charm to succeeding generations, isn't it the part of wisdom to leave nothing to chance or the possibility of errors in judgment?

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RALPH CLEON THOMAS

PHYTOPATHOLOGY

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RALPH CLEON THOMAS

1892–1928

S. A. WINGARD

Ralph Cleon Thomas was born at Grant, Grayson County, Virginia, April 9, 1892, and died at Tifton, Georgia, December 25, 1928. He was reared on a Grayson County farm and received his elementary education in the public schools of the neighborhood, graduating from the Bridle Creek High School, Bridle Creek, Virginia, in June, 1912.

Early in life he developed a love for the sciences, especially for botany. With his favorite science uppermost in mind he entered the Virginia Polytechnic Institute in 1913 and was graduated in June, 1917, receiving the B. S. degree in Agriculture. He was immediately appointed Student Assistant in the Department of Bacteriology and Plant Pathology of his Alma Mater. In this capacity he rendered proficient service and at the same time pursued graduate work in plant pathology until February 28, 1918, when he enlisted in the Medical Corps of the United States Army. He served in the bacteriological laboratory at Camp Lee, Virginia, until July, when he sailed with his unit to France. He served overseas until May, 1919.

Upon his return from Europe he received his discharge from the Army and resumed his graduate studies at the Virginia Polytechnic Institute and received the M. S. degree in plant pathology in July, 1919. He was immediately appointed Assistant County Agent in the Virginia Agricultural Extension Division to make a special survey of the nematode disease of wheat. He was appointed Plant Pathologist of the Virginia Agricultural Extension Division in November, 1919, in which capacity he served until September 1, 1922, working chiefly on cereal and tobacco diseases. Feeling the need of further training he went to Michigan State College as Graduate Assistant in Bacteriology and did special cultural and diagnostic work until August, 1923. He taught in the Jackson High School, Roanoke, Virginia, during the session of 1923–1924.

Being desirous of returning to research, his first love, he studied at Cornell University during the summer session of 1924. On October 15, 1924, he was appointed Assistant Plant Pathologist in the Office of To-

bacco Investigations, Bureau of Plant Industry, United States Department of Agriculture, and stationed at the Coastal Plains Experiment Station, Tifton, Georgia, where he conducted laboratory and field investigations on tobacco disease in the Southern States, more particularly in the new tobacco district of southern Georgia. This work dealt primarily with the development of methods of control for nematodes, black shank, wild fire, black fire, and other tobacco diseases of importance in the South. He was engaged in this work at the time of his untimely death.

My acquaintance with Mr. Thomas began in the fall of 1917 when I was employed by the Virginia Agricultural Experiment Station. We became intimate friends, roomed together for several years, and were closely associated in both work and play until the time of his death. I have never known a man who was more conscientious in the performance of his duties and more loyal and sincere to his friends than he. The genuineness of his of his friendship was indicative of his noble character. Among God's noblemen none ever was more merciful to his opponents; none more thoughtful of the unfortunate and neglected. He was a veritable incarnation of Christianity, and though taken in the flower of young manhood, Ralph Cleon Thomas will always be alive in the memory of his friends.

A STUDY OF THE BIOLOGY OF A NEW SPORE-FORMING RHIZOCTONIA, CORTICIUM PRATICOLA[1]

JOHN E. KOTILA[2]

INTRODUCTION

The fungi of the form-genus Rhizoctonia are among the most common and universally distributed groups of pathogenic soil fungi and have been studied by a great many investigators. As a result, much difference of opinion has arisen as to whether numerous species exist, each associated with certain hosts, or whether there are just a few main species, each of which may contain numerous forms or strains.

Matz (54) believes that there are a great many species and differentiates them by vegetative characters. Duggar (28) and Peltier (62), on the other hand, prefer to consider Rhizoctonia as composed of two main species, *Rhizoctonia solani* Kühn (*Corticium vagum* B. & C.) and *Rhizoctonia crocorum* (Pers.) DC. Briton-Jones (9), in his study of Rhizoctonia, found that differences occurred when a single strain was grown under different conditions and that, in his opinion, these differences were sufficiently marked to warrant separation of such a strain into distinct species, provided the Matz (54) method of differentiation be used. This investigator found macroscopic differences between such strains in culture but the microscopic differences were so slight that he did not think a multiplication of "species" justifiable on such grounds. He retained the name *Corticium vagum* (*Rhizoctonia solani*) for the forms or strains with which he worked, realizing, however, that the species contains several physiologic forms or strains. Gratz (34), Jochems (37), Matsumoto (53), Raeder, Hungerford, and Chapman (65), Thomas (79), and van der Meer (55) have reported strain differences in culture of *R. solani*. Thomas (79) studied 20 different cultures, obtained from various sources, and concluded from his examination of vegetative characters that all of them belonged to one botanical species, *R. solani*. He adds, however, that until it is possible to obtain in culture the basidiospore stage of the various strains, it will not be possible to classify them systematically.

It is common experience that *R. solani* remains sterile when grown on artificial media under ordinary laboratory conditions, while, in nature, the perfect stage of the fungus is formed more or less freely on a wide range

[1] Papers from the Department of Botany of the University of Michigan, No. 313.

[2] The author acknowledges his great indebtedness to Professor C. H. Kauffman, of the University of Michigan, under whose direction the present investigation has been conducted, and to Dr. E. A. Bessey and Dr. G. H. Coons, of the Michigan State College, for helpful suggestions and advice.

of hosts. Very little is known of the factors involved in the formation of basidiospores of *R. solani* and the lack of such knowledge has retarded the clearing up of the Rhizoctonia situation.

In 1924 the writer undertook an investigation to determine whether strains of *R. solani* and *R. crocorum*, such as those referred to above, have any significance. Cultures of Rhizoctonia were received from plant pathologists in various parts of the United States. Most of these cultures unquestionably belonged to the *R. solani* group. The *R. crocorum* group was not represented. A number of pure cultures of the latter were obtained, however, in October, 1924, while making a study of the violet root rot of alfalfa in Michigan. Two additional cultures of *R. crocorum* were received later from England through the courtesy of Buddin and Wakefield (11). All of these cultures formed the reddish brown or magenta colored mycelium characteristic of *R. crocorum*. At the same time a species of Rhizoctonia, apparently different from *R. solani* and *R. crocorum*, was isolated from a mass of hyphae and spores present on one of the diseased alfalfa plants. Several weeks later it was observed that this Rhizoctonia had produced its hymenial stage in pure culture (47). In view of the lack of information concerning the factors involved in spore formation in the Rhizoctonias and of the opportunity afforded by the new species to study this problem, it seemed advisable to undertake an intensive study of this new species.

The present investigation was begun and continued at the University of Michigan during the academic year, 1924–25, and has been continued at the Michigan State College up to the present time (1928).

HISTORICAL[3]

The first report of a plant disease caused by a species of Rhizoctonia was made by Duhamel (30) in 1728. His description of the symptoms indicates that the fungus concerned was *Rhizoctonia crocorum*. Although considered under different names, this fungus appears to have been the only form reported by investigators previous to 1858, when Kühn (48) described a different one, pathogenic on potato, and readily distinguishable from *R. crocorum*. Kühn named the new species *R. solani*. Considerable confusion later occurred with regard to these two species, and this led Duggar (28) to redescribe them and collate the synonymy of each.

Until 1903 basidiospores were not definitely associated with either species of Rhizoctonia, although many divergent views were held as to the perfect stage of *R. crocorum*. These are discussed fully by Duggar (28). Recently, however, Buddin and Wakefield (12) have reported *Helicobasidium pur-*

[3] Owing to the complete historical accounts of this group of fungi, already published by previous investigators (28, 59, 62, 74, 79), only those papers are considered which bear relation to the present study.

pureum (Tul.) Pat. intimately associated with *R. crocorum* and, from their study, they consider the balance of the evidence is in favor of the view that *Helicobasidium purpureum* is the perfect stage of *R. crocorum.*[4] Rolfs (72) in 1903 reported a species of Corticium as being definitely associated with *R. solani* and in 1904 (71) proved the relationship by obtaining the sterile Rhizoctonia stage from single spores of the basidiomycete stage. Burt (18) referred the perfect stage, discovered by Rolfs, to *Corticium vagum.* In 1891 Prillieux and Delacroix (64) discovered what they considered to be a fertile species of Hypochnus on a potato stem and named the fungus *Hypochnus solani.* These investigators, however, did not associate their Hypochnus with *Rhizoctonia solani,* already known to occur in Europe. Several European investigators (58, 63, 69) have since shown this identity to exist. American as well as some European investigators prefer to follow Burt's (16) classification of the Thelephoraceae in which the genera Hypochnus and Corticium are differentiated by certain basidiospore characters. Under Hypochnus, Burt places those resupinate species which have rough-wall to echinulate spores that are usually, but not always, colored, while those species with smooth-wall, hyaline, or colorless spores are classified under the genus Corticium. Both *Hypochnus solani* Prill. & Delacr. and *Corticium vagum* have colorless smooth-wall spores. The former, therefore, becomes a synonym of *C. vagum* according to the above classification; this interpretation is followed in the present paper. The Rhizoctonia stage of this species will hereafter be included in the binomial *Corticium vagum.*

Although *C. vagum* grows readily on artificial media, practically all investigators who have studied it have been unable to obtain the perfect stage of the fungus in culture. Thus Atkinson (2, p. 339–342), in discussing damping off of seedlings caused by a sterile fungus ("Rhizoctonia"), states: "Much of the trouble in the nature of damping off both in the forcing house and in the fields is caused by a fungus which has been under study at several different times during the last three years, but up to the present time (1895) has refused all the encouragement, which it has been possible from present experience to offer it, with the hope of inducing it to develop some characteristic fruiting organs, in order that its real nature and affinities might thus be known." Shaw (74) also had the same experience: "It was found impossible to get the perfect stage in cultures on artificial media. It is apparently strictly parasitic." In 1924 Briton-Jones (9) reported that while working in Egypt on sore shin of cotton caused by *Corticium vagum,* the fertile stage had been produced under

[4] An additional paper by these authors (13) appeared after this paper was ready for the press, bringing still more evidence of the genetic connection claimed in their first paper.

experimental conditions. Details of the experiment are not given in the report, but in a letter to the writer, he relates his observance of the Corticium stage on some peanut plants (*Arachis hypogaea* L.) growing in pots in the laboratory garden. These plants had been inoculated with *C. vagum* and had been placed in a shady spot where they were kept continually moist. Müller (59), during the same year, also reported that he obtained the hymenium of *C. vagum* on potato plants inoculated with single-spore cultures of the fungus. This investigator reported further (58) that he obtained the perfect stage in the laboratory by first growing the fungus in a rich nutrient solution and then, after a period of growth, transferring some of the mycelium to flasks containing filter paper saturated with water. Microscopic examinations were made daily after the second day. On the fifth day basidia and basidiospores were discovered in two of the cultures. The spore measurements corresponded to those of Saccardo (73, p. 130). Müller, unfortunately, was unable to duplicate the results obtained in the above experiment. However, his results are thought to represent the nearest approach to obtaining the perfect stage of a Rhizoctonia in artifical culture in the laboratory.

EXPERIMENTAL

Source of Material: Early in October, 1924, while making a study of the violet root rot of alfalfa, a number of diseased plants seriously affected with *Rhizoctonia crocorum* were obtained from an alfalfa field in Cass County, Michigan. These plants were replanted in eight inch pots in the greenhouse at the University of Michigan. The pots were filled with soil from the diseased area of the Cass County field and were watered regularly. They continued to grow in spite of the fact that the tap roots were enveloped in a heavy mycelial felt, characteristic of *R. crocorum* on this host.

On October 21, 1924, there was observed on one of the lower branches of one of the potted plants a white-appearing mass of fungous growth, about 5 mm. in diameter, which resembled the perfect stage of *Corticium vagum*. Anticipating the discovery of the perfect stage of *R. crocorum*, the branch was removed and the material examined under the microscope. Hymenial cells, basidia, and spores were observed.

Owing to the paucity of material, further study was sacrificed in order to pour plates to obtain the fungus in pure culture. Five isolations were made from two series of plates. The growth which followed was similar in all isolations but was white rather than reddish brown or violet as would be expected in *R. crocorum*. Apparently the alfalfa plant from which the isolations were made was affected with two species of Rhizoctonia which the writer believes to be genetically distinct. At no time since its isolation has the new form of Rhizoctonia produced growth that could be mistaken for

that of *R. crocorum*; several pure cultures of the latter were made at about the same time from some diseased alfalfa plants obtained from the same field.

The five isolations made from the mass of hymenial material grew vigorously on Leonian's (49) agar and, after several weeks, it was observed that four of the cultures produced basidiospores while, curiously, one of them, under like conditions, seemingly remained sterile. One of the spore-forming cultures was selected for further study and, for convenience, was designated R-40. The sterile form was numbered R-43.

Following the isolation of this spore-forming Rhizoctonia, a careful examination was made of cultures received from plant pathologists in various parts of the United States. Other cultures isolated from various hosts in Michigan were examined also to ascertain whether they resembled the new Rhizoctonia. Three were found. Two had been isolated from alfalfa and one from bean. One of the alfalfa isolations (R-22) was made by the writer from a damped-off alfalfa seedling grown in a flat of diseased soil obtained from the Cass County field referred to above. The other culture (R-38) was isolated from the roots of an alfalfa plant in Minnesota and was sent to the writer through the courtesy of E. C. Stakman. The third culture (R-44), which somewhat resembled the spore-forming Rhizoctonia, was isolated by the author from a bean which had failed to germinate when planted in wet sphagnum. When grown under similar conditions on Leonian's agar, it was found that of these three only the Minnesota fungus formed spores. The mycelium, hymenial cells, basidia, sterigmata, and basidiospores produced by this fungus were identical with those of the original spore-forming Rhizoctonia isolated by the writer.

Description of the Spore-forming Rhizoctonia. Microscopic examination established the fact that, although the mycelium and sclerotia of this Rhizoctonia are somewhat similar to those of *C. vagum*, the basidia are markedly different. Macroscopic examination also revealed other differences, especially as to color and the abundance of sclerotia. The mycelium of the fungi isolated from alfalfa was white as contrasted with the brown mycelium of *R. solani*; and the sclerotia were neither so large nor so many as those usually formed by the latter fungus. These differences were especially evident when the two fungi were grown on potato-dextrose agar. (Fig. 1.)

The new spore-forming Rhizoctonia belongs to the Basidiomycetes, as is shown by the fact that it forms true basidia, each of which forms 1 to 4 sterigmata. Each of these, in turn, bears a basidiospore.

The outstanding characteristic of the basidia of this fungus is the unusual length of the sterigmata. Actual measurements of these have varied from 13 to 26.5 μ, with an average of 18.8 μ. (Fig. 5, A, and Fig. 6.) The

sterigmata taper gradually from the point of attachment at the basidium to the point of attachment at the basidiospore, the greatest diameter being approximately 2.6 μ. The spores are hyaline, ovate, apiculate, and smooth-wall, having an average size of 7.7×5.2 μ. The average dimensions of the hyaline basidia are 15.6×6.5 μ. No cystidia have been observed. The basidia are arranged in a primitive hymenium, *i.e.*, many small areas, interrupted by sterile areas, are scattered over the surface of the culture in more or less abundance. No continuous fructification of a size easily visible to the eye was ever observed.

From the fact that the hymenium is resupinate, the basidiospores glabrous and hyaline, and no cystidia are present, it would seem that the fungus belongs to the genus Corticium. As *Corticium vagum* was shown by Rolfs (72) to be the perfect stage of *Rhizoctonia solani,* it might be considered

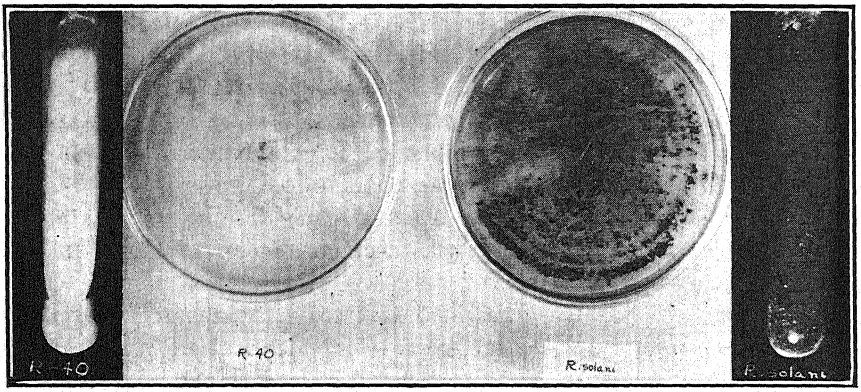


FIG. 1. *Corticium praticola* sp. nov. left, and *C. vagum* B. & C. right, on potato-dextrose agar showing differences in vegetative growth.

that, owing to the somewhat similar vegetative growth of this new spore-forming alfalfa species, its perfect stage can be referred to a position close to the former. However, there appear to be marked morphological differences between the descriptions of *C. vagum* and that given above. The spore dimensions of the alfalfa fungus are much smaller than those of *C. vagum,* for which Burt (18) gives $8–14 \times 4–6$ μ. This mycologist describes *C. vagum* as having 4 to 6 sterigmata, varying in length from 6 μ to 10 μ, while the sterigmata produced by the alfalfa species vary in number from 1 to 4, and vary in length, both in culture and on artificially inoculated alfalfa plants, from 13 to 26 or more microns. (Fig. 5, A, and Fig. 6.) In all illustrations of *C. vagum* examined by the writer (18, 25, 32, p. 115; 54, 59, 64, 74), the basidiospores are shown to be attached to the basidia by sterigmata which are very short, and they are seldom pictured as greater than the length of

the spore (74) and often shown as less than half of the spore length (18). (Fig. 5, C, D, E.) Numerous instances have been observed in the case of the new alfalfa Rhizoctonia in which the sterigmata measured more than three times the length of the greatest diameter of the basidiospore.

Until it can be shown that the perfect stage of *C. vagum,* when produced on artificial media, is similar to that described for the new alfalfa pathogene, there seems to be sufficient morphological evidence to warrant classification of this fungus as a new species of Corticium. The name **Corticium praticola,** sp. nov., is therefore proposed.

CORTICIUM PRATICOLA KOTILA, SP. NOV.

Vegetative hyphae hyaline, Rhizoctonia-like, 6 to 8 μ in diameter, septate with septa at 80 to 100 μ intervals. Sclerotia on artificial media brown, and, in general, smaller and less abundant than those formed by *Corticium vagum.* Sclerotial cells variable in size and shape. Hymenium loose, in minute placques formed by a more or less dichotomous branching of the vegetative hyphae. Terminal cells develop into basidia bearing 1 to 4 sterigmata, the usual number being 3. Basidia average 15.6 μ long and 6.5 μ wide. Sterigmata vary from 13 to 26.5 or more μ long, average 18.8 μ, and taper gradually upward from point of attachment to basidium, greatest diameter 2.6 μ. Basidiospores hyaline, ovate, apiculate, smooth-wall, from 5.2 to 7.8 μ long and from 4.9 to 5.5 μ wide, with average dimensions of 7.7 by 5.2 μ. Cystidia lacking.

Type cultivated in the laboratory; obtained in Michigan. Saprophytic in soil and parasitic on *Medicago sativa* L. in cultivated fields, collected by J. E. Kotila, October, 1924.

Reinoculation of Host. It was not difficult to infect alfalfa with pure cultures of the new Corticium and to recover the fungus from the infected plants.

In several experiments, conducted in the greenhouse during the winter 1924–25, one- or two-year-old alfalfa plants growing in pots were inoculated with pure cultures of the fungus, R–40. After a period of 30 days it was found that infection had taken place. No indication of the perfect stage was observed on these plants. Later (1926), having learned that humidity is a controlling factor for basidiospore formation, the inoculation experiments were repeated.

In one of these repetitions alfalfa seedlings 2–4 mm. in diameter were disinfected in mercuric chloride, 1:1000, for two minutes, and then planted in small pots of soil. The pots and soil had previously been steam-sterilized three hours in an Arnold steamer. The soil of 9 pots was inoculated with R–40, while that of a like number was inoculated with the apparently sterile

R-43. After inoculation the pots were placed in a Wardian case in the greenhouse. The floor of this case was covered with wet excelsior in order to make the humidity greater than that of the air in the general greenhouse. After a period of two weeks the data shown in table 1 were obtained.

TABLE 1.—*Reinoculation of alfalfa with Corticium praticola*

Isolation No.	No. of pots	No. of plants	No. plants infected	No. showing perfect stage
R-40 (Fertile)	9	9	9	3
R-43 (Sterile)	9	9	9	0

The basidia, sterigmata, and basidiospores formed upon the three plants which showed the perfect stage, were found to be identical with similar structures formed on artificial media. Both the numbers were reisolated from the infected plants without any apparent change in the character of the fungus. The fertile one formed its basidiospores typically and abundantly, while the other remained apparently sterile.

This experiment was repeated, but, instead of using the Wardian case, the pots were placed in a small, galvanized-iron, glass-cover box near a window in the laboratory. The floor of the box was covered with wet excelsior. Owing to the box being only of slightly larger capacity than was needed for the pots, a higher humidity was obtained than was possible in the Wardian case of the preceding experiment. The results obtained from this trial, shown in table 2, were recorded three weeks after inoculation.

TABLE 2.—*Reinoculation of alfalfa with the C. praticola under conditions of high humidity*

Isolation No.	No. of pots	No. of plants	No. plants infected	No. showing perfect stage
R-40 (Fertile)	5	10	10	10
R-43 (Sterile)	5	10	10	0

As was the case in the preceding experiment, the hymenial cells, basidia, sterigmata, and basidiospores were similar to those obtained on artificial media.

Growth and Reproduction under Controlled Conditions. The establishment of fundamental principles underlying the control of growth and reproduction in plants was due largely to the pioneer work of Klebs (40). This investigator showed the relative importance of external conditions, such as food, moisture, heat, light, oxygen, etc., and their influence on growth and the reproductive processes of plants; he determined how certain of these

factors acted, and was able at will to cause the organisms which he had under observation to remain in the vegetative condition or to proceed to the reproductive phase. Klebs (41) believed that the external factors determined whether growth or reproduction was to take place, the internal factors, in turn, responding to the stimuli exerted by the external environment.

In the experiments reported in the following pages the object has not been to confirm the work of the above investigator. On the contrary, the end in view has been to apply the principles of Klebs to the organism under consideration in order to determine optimum conditions for formation of basidiospores in this species of Corticium.

It is a well-known principle that reproduction in fungi is preceded ordinarily by vigorous vegetative growth. It was, therefore, necessary to learn what were the optimum conditions for vigorous mycelial development before proceeding to determine the optimum conditions for reproduction. These experiments follow.

Food. Early in the work a study was made of the effect of various sugars, including maltose, levulose, glucose, and sucrose, upon the mycelial growth of the spore-forming Corticium. It was learned from this study that the mycelium developed about equally well on any of these sugars. Because maltose appeared to favor the growth in some other cultures of Rhizoctonia, which were being studied at the same time, this sugar was selected as the source of carbon in most of the media employed in the present work.

Inorganic Salts. The favorable effect of potassium dihydrogen phosphate, calcium nitrate, and magnesium sulphate on the growth of fungi having been shown by many investigators, these chemicals were tested out in various amounts to determine the most favorable concentration for the vegetative growth of the Corticium being studied. Coons (22), Leonian (49), and Bonar (8) found certain concentrations of these chemicals to be most favorable for the growth of the fungi which they had under observation. Using as a starting point the concentration found to be favorable by the above investigators in their study of other fungi, a stock solution was prepared according to the following formula:

Potassium dihydrogen phosphate	M/5	4.60 cc.
Calcium nitrate	"	1.28 cc.
Magnesium sulphate	"	1.27 cc.
Maltose	"	8.60 cc.
Agar		2.00 gm.
Water (distilled) to make		100.00 cc.

The concentration of the chemicals used in this formula is considered 1x. In the following experiment the concentration of the chemicals was varied one

at a time, the others remaining at 1x. Concentrations of 1x, 2x, and 4x of each chemical were tried out in 10 cc. quantities in test tubes and the trials were conducted in duplicate. The cultures were grown at room temperature for sixty days. The effect of the concentrations of the above chemicals upon the development of the mycelium of the new Corticium is shown in table 3.

TABLE 3.—*Effect of concentration of various chemicals upon the growth of Rhizoctonia (R-40)* (*Scler.* = *sclerotia*)

Chemical	Days at 25° C.	Concentration					
		1x		2x		4x	
		Mycel.	Scler.	Mycel.	Scler.	Mycel.	Scler.
Potassium dihydrogen phosphate	7	I[4]		III	II	III	
	10	II		II	I	III	I
	14	II–		II–	I	II	I
	60	II		II	I	III	I
Calcium nitrate	7	I		III	II	III	II
	10	II		III	II	III	II
	14	II–		II	I	II	I
	60	II		III	I	III	I
Magnesium sulphate	7	I		II		III	
	10	II		II		II	
	14	II–		II	I	II	I
	60	II		III	I	III	I

[4] Growth: I–poor; II–fair; III–good.

In a week after the test tubes were inoculated the cultures had extended over the entire surface of the media, thus obscuring the accuracy of the readings after that period. Observations made 10 days after the experiment had been started indicated that the higher concentrations produced only slightly better growth than the 1x in the case of potassium dihydrogen phosphate and magnesium sulphate. In the case of the calcium nitrate, however, the increased growth in the 2x and 4x concentrations was especially pronounced.

Organic Nitrogen. Of the various carriers of nitrogen only the more common forms, malt extract, peptone, and asparagin were compared. Of the three, malt extract was found to cause by far the most abundant mycelial development.

Using the results obtained in the preceding experiments, the concentrations of the various substances most favorable for growth were selected and a medium was prepared according to the following formula:

Potassium dihydrogen phosphate	1.25 gm.
Calcium nitrate	2.36 gm.
Magnesium sulphate	0.59 gm.
Maltose	6.25 gm.
Malt extract	6.25 gm.
Agar	20.00 gm.
Distilled water to make	1000.00 c.c.

Autoclave at 10 pounds for 15 minutes.

The Corticium under consideration grew well on this medium and formed spores very abundantly. With few exceptions, this medium was used in all of the experiments in relation to environmental factors. Coons' (22) synthetic medium, because of its greater transparency, was found superior when it was necessary to make microscopic observations through inverted petri dishes. This medium also was used in that part of the present investigation dealing with the isolation of single spores from basidia while under constant observation through the microscope.

Having obtained a medium highly favorable for vegetative growth, the following experiments were conducted to determine optimum conditions for the formation of basidiospores.

Moisture. The effect of humidity on spore formation of R-40 was determined by a series of experiments in which cultures were grown in test tubes of malt-extract agar, prepared according to the foregoing formula. The cultures were grown at room temperature in incubators provided with glass doors. A range of humidity was obtained by placing pans of water in the incubators. The check cultures were grown on a laboratory table adjacent to the incubators. Humidity readings were made by means of Lambrecht polymeters. A typical range of humidity and the effect upon basidiospore formation obtained in these experiments are shown in table 4.

TABLE 4.—*Effect of humidity upon spore formation in Rhizoctonia R-40 (C. praticola)*

Place	Humidity percentage	Basidiospore formation
Incubator No. 1	90.0	Meager
" " 2	82.0	Fair
" " 3	65.0	Abundant
Laboratory table	32.0	None

It is obvious that the moisture content of the air is a very important factor in basidiospore formation in *C. praticola.* The above results confirm field observations which have shown that the Corticium stage of *R. solani* on potato stems is more prevalent during seasons of abundant rainfall and consequent high atmospheric humidity than in comparatively dry

seasons. The correlation of abundant moisture with the perfect stage of this fungus has been recognized by many investigators (9, 62, 74).

Temperature. Richards (66, 67, 68) showed that the *C. vagum* studied by him grew within a temperature rage of 4.6° to 32.6° C., the optimum being between 25° and 27° C. He also found that the soil temperature influenced the pathogenicity of the fungus and that the optimum temperature for tissue destruction on potato, pea, bean, and cotton was approximately 18° C. Müller (59) reported a temperature range of 4.5° to 30.8° C. for the *Hypochnus solani* (*R. solani*) he had under observation. The latter investigator found 24° C. to be the optimum temperature for growth of his organism.

The effect of temperature on vegetative growth and basidiospore formation of the species of Corticium studied by the writer was determined in two experiments. Duplicate cultures in test tubes were grown at various temperatures obtained in the compartments of a differential thermostat, one end of which was cooled in a tank containing ice and brine, while the other end was heated by means of an electric-light bulb. In the first experiment a range of temperature from 9.6° to 34.1° C. was obtained in the seven compartments of the thermostat. The cultures were grown for two weeks when the following observations were made:

Very feeble growth had occurred at the minimum and maximum temperatures.

Optimum growth occurred at 19° to 21° C.

No provision was made to maintain humidity in the compartments of the thermostat in this experiment, hence no basidiospores were formed.

TABLE 5.—*Effect of temperature upon growth and basidiospore formation of Rhizoctonia (R-40). (C. praticola)*

Temperature[5] (Centigrade)	Mycelium	Sclerotia	Basidiospores
2.1	0	0	0
9.9	I[6]	0	0
16.0	III	IV	I
18.6	IV	III	II
20.7	IV	II	III
24.3	V	I	I
35.2	II	0	0

[5] Average daily temperature for 30 days.

[6] No growth, sclerotia, or spores. 0
Growth poor, sclerotia and spores few I
Growth excellent, scler. and spores many V

In repeating the experiment a higher humidity was obtained by placing absorbent cotton, saturated with water, in each compartment and by the addition of water from time to time as required. The temperatures were recorded daily and, after 30 days, the cultures were removed and the data, shown in table 5, were obtained.

As shown in table 5, the optimum temperature for mycelial growth is 24.3° C. which is very close to that reported by Müller working with *R. solani*. However, the differences in growth between 18.6° and 24.3° C. were not very marked. The temperature range for sclerotium and basidiospore formation was found to be the same (16.0° to 24.3° C.). The optima, however, were different. Sclerotia were most numerous at 16° C., while basidiospores were most abundant at 20.7° C.

Light. In nature the perfect stage of *C. vagum* is found at the base of the stems of the plants on which it occurs and sometimes it extends 6 to 20 cm. up the stem or out along the first branches. While the light is somewhat subdued in most situations where *C. vagum* is found, occasionally the fruiting stage is observed under fully exposed conditions. In order to determine whether light is essential to the formation of the perfect stage of *C. praticola,* the following experiment was performed. Twenty test tubes containing malt-extract agar were inoculated with transfers from a vigorously-growing culture of Rhizoctonia (R-40). Ten cultures were placed in a test-tube rack in a light-proof box. The ten remaining cultures were placed in a similar test-tube rack exposed to light. Both sets of cultures were placed in an incubator having a humidity of approximately 90 per cent and a temperature of 24° C. The incubator was placed about eight feet from an east window. A 500-Watt-electric light was placed near the glass door of the incubator so that daylight could be supplemented by electric light on cloudy days. As in previous experiments, the cultures were allowed to grow for 30 days before the data shown in table 6 were obtained.

It was observed during the course of the experiment that those cultures nearest the source of light were the first to form spores and, at the conclusion of the test, basidiospores were more abundant in the five cultures nearer the source of light than in the five farther removed (Cultures Nos. 16 to 20). It is interesting to note that sclerotium formation occurred only in the cultures kept in the dark.

Some basidiospores were formed under conditions of darkness, but it would appear that light strongly stimulates spore formation.

Reaction of the medium. Although many fungi are known to tolerate a wide range of hydrogen-ion concentration in the substrata upon which they grow, some are known to be more or less restricted. *Actinomyces scabies* (Thax.) Güssow has long been associated with alkaline soils, and it is common knowledge amongst potato growers that common scab does not

TABLE 6.—*Effect of light upon basidiospore production of Rhizoctonia (R-40). (C. praticola)*

Culture No.	Conditions	Basidiospores	Sclerotia
1	Light	III[7]	0
2	"	III	0
3	"	III	0
4	"	III	0
5	"	III	0
6	Dark	I	I
7	"	I	I
8	"	I	I
9	"	I	I
10	"	I	I
11	"	I	I
12	"	I	I
13	"	I	I
14	"	I	I
15	"	I	I
16	Light	II	0
17	"	II	0
18	"	II	0
19	"	II	0
20	"	II	0

[7] Basidiospores abundant III
" meager I

occur on potatoes on acid soils. Gillespie (33) showed that the growth of this potato-scab organism was inhibited at a reaction of pH 5.2 or less. Whereas, common scab of potatoes is limited to nonacid soils, the disease caused by Rhizoctonia is known to occur on potato plants grown on a great variety of soil types irrespective of hydrogen-ion concentration of such soils. Likewise, the *C. vagum* stage has been observed under a wide range of these conditions. The tolerance shown by *R. solani* in nature was found to hold in the case of Rhizoctonia R-40 when grown on artificial media.

Coons' synthetic medium, in test tubes, was adjusted colorimetrically to various hydrogen-ion concentrations, ranging from pH 4.0 to pH 7.6, by the addition of N/10 hydrochloric acid or N/10 sodium hydroxide. The unadjusted medium had a reaction of pH 5.2. Three tubes adjusted to each reaction were inoculated and the cultures grown for 14 days at 24° C. in an incubator having a humidity of approximately 60 per cent.

The reaction of the medium within the range tested (Table 7) had no effect either on the growth of mycelium or on the formation of basidiospores in the case of the fungus under consideration. The results obtained

were so uniform that the effect of the reaction of the substratum on the fungus was no further studied.

TABLE 7.—*Effect of reaction of substratum upon basidiospore formation of Rhizoctonia R-40. (C. praticola)*

Reaction	Basidiospore formation	
	8 days	14 days
pH 4.0	II[s]	III
4.6	II	III
5.2	II	III
6.2	II	III
7.6	II	III

[s] Basidiospores few I
" numerous III

Oxygen. In an earlier study of *R. solani,* it was learned that an abundant supply of air was required and that the fungus did not remain alive for more than 4 days in cultures kept sealed in test tubes of 30 cc. capacity. In the present study of Rhizoctonia R-40, it was noticed that basidiospores in test-tube cultures were first formed on that part of the medium near the tip of the slant. In petri-dish cultures, the hymenial cells formed first and their basidiospores occurred on the outer edge of the inner surface of the top plate nearest the source of air.

Klebs (41) was of the opinion that oxygen exerted little or no influence on the appearance of reproductive stages of *Sporodinia grandis* Link. and showed that transpiration was the controlling factor. In order to determine more definitely what influence oxygen had upon the growth and basidiospore formation of *C. praticola,* several experiments were carried out as follows:

Cultures were made on Coons' synthetic agar in petri dishes. The cultures were placed on pieces of window glass and covered with large bell jars fitted with two-hole rubber stoppers. Short pieces of glass tubing were inserted into the stoppers and to one was atached a piece of rubber tubing which extended to the bottom of the bell jar. Air under pressure was forced through one bell jar continuously. The air of the second bell jar was allowed to diffuse in and out unassisted. Abundant humidity was supplied in both bell jars by means of absorbent cotton saturated with water. After two weeks' growth it was observed that basidiospores had formed very abundantly in the aerated cultures and very feebly in the nonaerated set.

A second experiment was performed in which petri dishes, inoculated as before, were placed in two Novy jars. Air under pressure was passed through one of the jars for 15 minutes daily after which the jar was sealed. Oxygen[9] was passed through the other jar each day for a similar length of time after which the jar was likewise sealed. After two weeks the Novy jars were opened and the cultures examined. The aerated cultures again had formed hymenial cells and basidiospores abundantly, while the oxygenated cultures were like those of the nonaerated set of the preceding experiment and indicated that an atmosphere containing a high percentage of oxygen is not favorable for basidiospore formation in the fungus concerned.

The results obtained in the above experiments seem to indicate that a study of the respiration quotients of this fungus would yield interesting results. However, such an extensive undertaking, as reported by Novy, Roehm, and Soule (61) was not attempted in the present study.

INHERITED FACTORS

From the preceding experiments it follows that several different external conditions take an important part in the control of basidiospore formation. Abundant humidity, a proper temperature, an optimal oxygen supply, and a determined food quality are necessary for their best development. Under these optimum conditions the spore-forming units developed and produced many spores. However, when other isolated units of similar origin were grown under the same conditions, they remained in the vegetative stage, indicating that, in addition to external conditions, certain inherited potentialities are placed differently in the different spores produced by this otherwise fertile fungus (R-40).

Heterothallism. The occurrence of heterothallism in fungi was reported first in the Phycomycetes by Blakeslee (6, p. 319). Considerably later the phenomenon was found present in the Basidiomycetes by Bensaude (5), Kniep (45), Buller (14), Mounce (57), Hanna (35), Newton (60), and others. Heterothallism has also been demonstrated in the Ascomycetes by Dodge (27) and by Shear and Dodge (75); in the Ustilaginales by Kniep (46) and by Stakman and Christensen (77); and by Craigie (23) in the Uredinales.

Müller (59), basing his opinion on the behavior of single-spore cultures inoculated on potato plants growing in pots in the greenhouse, concluded that "*Hypochnus solani*" (*C. vagum*) is homothallic.

In describing the isolation of *Corticium praticola* mention was made of the fact that four of the five isolations made by the writer formed

[9] The oxygen used was a product of the Air Reduction Sales Co., Detroit, Michigan, whose analysis showed the gas to be 99.5 per cent oxygen.

basidiospores in culture, while, curiously, one remained apparently sterile. It was related further that search through the stock cultures of the Rhizoctonias on hand revealed two more apparently similar ones which likewise remained sterile. These three sterile cultures have been designated as R-43, R-22, and R-44, respectively, while the fertile strain heretofore referred to as R-40 was selected for comparative study. In attempting an explanation of the behavior of the first five isolations made from the original mass of hymenial cells and basidiospores obtained from the alfalfa stem, the following hypothesis presented itself: That the four spore-forming isolations had come from bits of hymenial material which had one kind of sex value, while the seemingly sterile offshoot was possibly a single-spore isolation of a different sex value. Such an hypothesis would assume that the fungus is heterothallic.

In order to test the validity of the hypothesis a preliminary experiment was performed in which the apparently sterile form R-43 was grown in the presence of R-22 and R-44. The latter cultures also were grown together. The matched cultures were grown at room temperature in small capsules of approximately 25 cc. capacity, each of which contained 10 cc. of malt-extract agar. The cultures were kept on a laboratory table about five feet distant from a north window. A month later microscopic examination showed that basidia, sterigmata, and spores, similar to those formed by R-40, were present in the capsules containing the cultures arranged thus: R-43 x R-22 and R-43 x R-44 but none were found in the capsule in which the cultures were matched as follows: R-22 x R-44.

The interesting results obtained in the preliminary experiment led to a more comprehensive confirmatory test. The three apparently sterile forms were again matched in all possible combinations. The cultures were grown in capsules as before and observations were made after 30 and 45 days. The data obtained from this experiment (see Table 8) were not so conclusive as one might expect from the results of the preliminary experiment. Basidiospores were formed only in one culture containing the matched forms and, as was the case in the first trial, the basidiospores occurred in only a small area. The mycelia of the two fungi intermingled and there was no microscopic evidence to indicate conjugation such as has been described by Blakeslee (6, p. 319).

The experiment was repeated numerous times but in only one additional case were basidiospores formed, when R-43 was grown in the presence of another one of these forms. Here again spore formation was not abundant. In all of the experiments the check R-40 formed a hymenium and basidiospores abundantly over the entire surface of the mycelial base as well as on the mycelium which had grown up to the sides of the containers.

TABLE 8.—*The results obtained by matching possible sexual strains of Corticium praticola*

Mating	No. of cultures	Results			
		30 days		45 days	
		Pos.	Neg.	Pos.	Neg.
22 x 22	10	0	10	0	10
22 x 43	10	0	10	0	10
43 x 43	10	0	10	0	10
43 x 44	10	1[10]	9	1[11]	9
44 x 22	10	0	10	0	10
44 x 44	10	0	10	0	10
40 x 40 (CK)	1	1	0	1	0

[10] Hymenial cells only.

[11] Basidiospores.

Such evidence as was obtained from the above experiments was, of course, inconclusive. Thinking that stronger proof of heterothallism might be obtained by mating single-spore cultures of the spore-forming Rhizoctonia R-40, twenty such cultures were made. These single-spore isolations were made by the Kauffman (39) method of distributing a dilute spore suspension by means of a fine pipette over the surface of a previously poured and hardened plate of malt-extract agar. Single spore were then cut out. Of the twenty single-spore isolations obtained, two formed basidiospores similar to and as abundantly as the parent culture. The eighteen which were apparently sterile could be classified into two groups according to rate of growth. Representatives of these two groups are shown in figure 2 and in figure 5, F. The differences in type of growth were very evident during the first few days after transfers were made. However, after the mycelia of the slow-growing cultures had covered the surface of the medium, macroscopic differences were no longer striking. It is interesting to note that Müller (59) observed differences both in the rate of growth and in the virulence of the single-spore cultures of *Corticium vagum* (*Rhizoctonia solani*) which he had under observation.

The sterile single-spore forms of Rhizoctonia R-40 were grown together in various combinations under conditions known to favor spore formation in the parent culture. No spores were observed in any of the matched cultures. Finally, all of the apparently sterile single-spore forms were matched in all possible ways, as shown in table 9. The experiment was performed in duplicate and four capsules were inoculated from the parent R-40 culture as a check on the favorableness of the external environment for basidiospore formation.

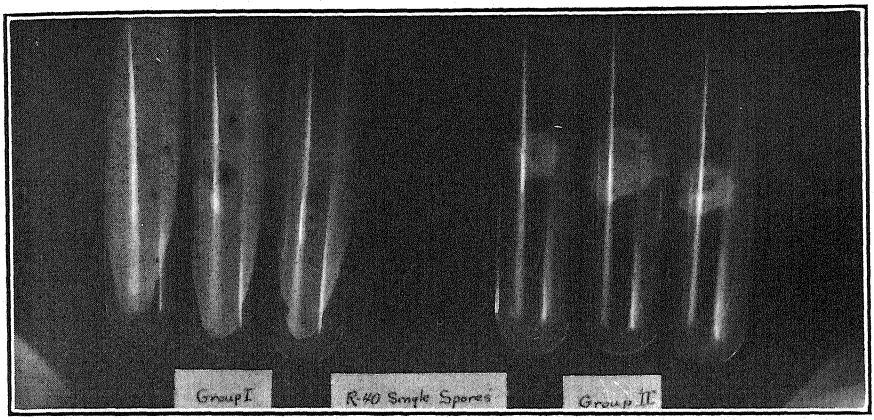


FIG. 2. Groups of rapid- and slow-growing single-spore cultures of *C. praticola*.

TABLE 9.—*Effect of matching single-spore cultures of Rhizoctonia R-40*

		Group I										Group II							
		Rapid growers										Slow growers							
		1	2	3	4	5	6	7	14	18	20	8	9	10	11	12	13	16	19
Group I	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	14	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	18	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Group II	8	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	11	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	12	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	13	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	16	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

The cultures were grown 30 days and then examined with hand lens for evidence of basidiospores. All suspicious cultures were reexamined under the microscope. In no case were basidiospores observed except in the check cultures and in these spore formation could easily be detected by the experienced, even if unaided, eye.

Hanna (35) has shown that in the four-spore *Coprinus lagopus* Fr. sex is determined by certain factors which segregate according to Mendelian principles, and that single-spore mycelia from any individual fruit body of this fungus belong to four sexual groups. Complete fertility results when union occurs between any two mycelia lacking a common factor. In the light of Hanna's results, it might be assumed that the failure to obtain the perfect stage in the matching experiments described above was due to the fact that all of the single-spore forms possessed some common factor. In order to determine whether this was actually the case, it was necessary to isolate single basidiospores from individual basidia and to determine the behavior of these single-spore cultures when grown alone and with each other in various combinations.

The dry-needle method of making monosporous cultures of Hymenomycetes and other fungi described by Hanna (36) is not applicable to fungi which produce a loose hymenium as is the case in *Corticium praticola*. The small size of the spores, their hyaline color, and the lack of uniformity in the number of spores formed by the basidia all indicate the unreliableness of any method of spore isolation from single basidia in which the basidia and spores are not under constant observation during the process of isolation.

Isolation of single spores of Corticium praticola (R-40) from individual basidia. The basidiospores of the form R-40 are formed on long sterigmata which extend out from the basidium in such manner that the individual spores are usually, relatively considered, at a considerable distance apart from each other. This fortunate position of the spores suggested the possibility of their isolation by using the technique reported by Barber (3) and Chambers (20) for the isolation of single bacterial cells.

After numerous preliminary trials the following technique was adopted. Cultures of the fungus were grown in petri dishes on Coons' synthetic agar under environmental conditions favoring spore formation. The medium was sterilized by autoclaving instead of by steaming on three successive days.[12] After 14 days hymenial cells, basidia, and spores were usually found to be abundant and were formed near the surface of the medium. A petri-dish culture containing basidiospores was placed, with the top plate removed, on the stage of a microscope to which was attached a "Barber micropipette manipulator," equipped with two glass needles. The diameter

[12] Coons, in conversation with the writer, expressed his belief that sterilization of his medium by autoclaving instead of by steaming on three successive days causes some change in the medium which is not favorable for mycelial growth. The suppression of mycelial development is, in this method of spore isolation, an advantage in that much of the difficulty incident to tangling of mycelia on the needles and loss of spores already picked is eliminated.

at the points of these needles was approximately ten microns.[13] Rapid drying-out and collapsing of the hymenial cells and basidia in the petri dishes was prevented by placing the microscope in a moist chamber. The humidity of the atmosphere of the small culture room, in which the isolations were made, was further increased by keeping the floor wet. Illumination was provided by a small electric microscope lamp placed directly under the condenser of the microscope. Heat from the light was absorbed by water contained in a flat bottle placed directly over the lamp. The low-power 16 mm. objective and No. 10 ocular were used in the isolation of single basidiospores. This was accomplished by first lowering the points of the needles, previously centered under the microscope, to a point approximately 5 mm. above the surface of the medium. The objective of the microscope was then lowered until the points of the needles were in focus after which it was again lowered until the hymenial cells, basidia, sterigmata, and spores could be seen. The petri dish was next moved about until a fungus-free area was found. The needles were were cleaned by lowering the points into the agar of this clear area and by forcing them through the medium in various directions by means of the lateral and longitudinal adjustments of the micropipette manipulator. After cleaning, the needles were raised so that the points were above the hymenial cells and the aerial portions of the fungus. The petri dish was moved about until a basidium was found on which all of the spores could be seen. (Fig. 6.) One of the needles was then lowered and by manipulation of the various adjustments was brought into contact with a basidiospore. On drawing the needle away the mature spore could be seen adhering to the needle. (Fig. 6, D.) By further manipulation a second and sometimes a third spore were picked on the same needle at some distance apart. At times both needles were used, picking one basidiospore on each in the case of a two-spore basidium, or one on one needle and two on the other in the case of a three-spore basidium, or two on each when the spores were picked from a four-spore basidium. After the spores had been removed from a basidium, the objective of the microscope and the needles were raised so that the petri dish containing the culture could be removed and replaced by another containing the same medium. The bottom plate of this dish had been previously marked as illustrated in figure 3. The needles were lowered again to a point about 5 mm. above the surface of the medium and the objective of the microscope was focussed downward until the needles were visible. The presence of the basidiospores was checked. The petri dish was then moved about until the desired marked area was centered under the microscope. The location was confirmed by focussing down through the medium, after which the objective

[13] The technique reported by Barber (3) was utilized in the preparation of the microneedles used in the present investigation.

was raised to a point slightly above the surface of the agar. One of the needles bearing basidiospores was then lowered until it could be seen. The objective and needle were lowered alternately until the surface of the medium was in focus. The needle was then lowered so as to come into contact with the medium. The film of moisture on the surface of the medium released the basidiospore from the needle, raised immediately afterward to

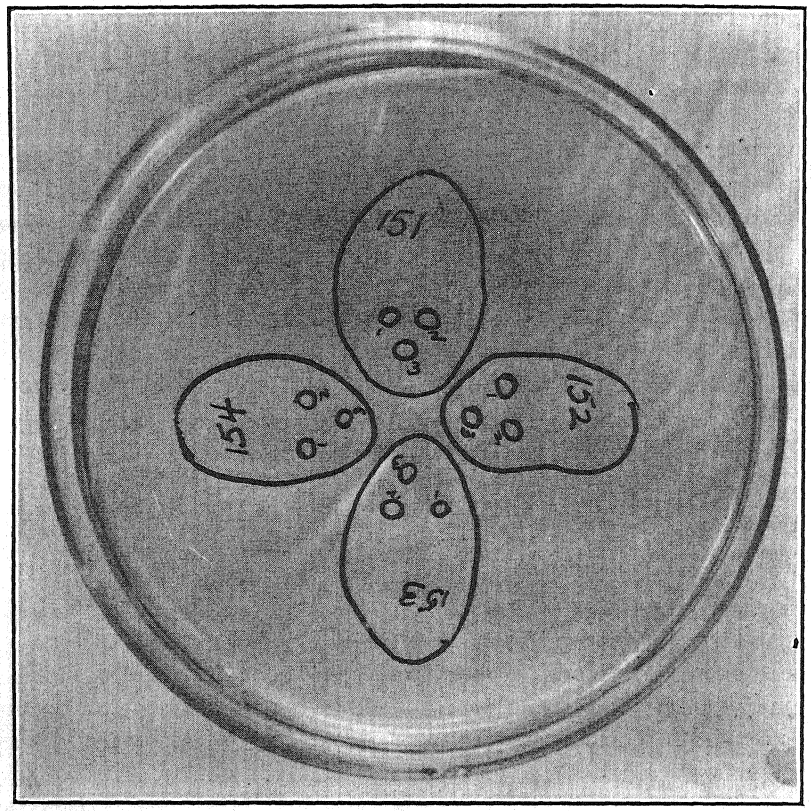


FIG. 3. Isolation of single spores. Method of marking petri dishes.

prevent a second spore from being deposited in the same area. A slight manipulation of the fine adjustment of the microscope revealed the basidiospore lodged on the surface of the medium. The petri dish was then moved so that another marked area was centered upon the microscope and another basidiospore was planted like the first, and the process was repeated for the other spores which had been isolated from the same basidium. After planting the basidiospores, the objective of the microscope and the needles were raised and the petri dish exchanged for the one containing the culture and

the operation of picking basidiospores was repeated. After becoming accustomed to the manipulation of the various adjustments of the pipette manipulator basidiospores could be picked from the one dish and planted in another in a relatively short time.

The petri dishes containing the planted basidiospores were examined microscopically through the bottom of the plates after 24 hours for evidence of germinating spores and after 48 hours all that had germinated were transplanted to test tubes containing slants of Coons' synthetic-agar

TABLE 10.—*Behavior of single-spore cultures isolated from the spore-forming Rhizoctonia R-40. (C. praticola)*

Basidium No.	Number of spores				Behavior	
	Formed	Isolated	Germinated	Grew	Fertile	Sterile
6	3	3	2	2	1	1
7	2	2	2	2	2	0
10	2	1	1	1	1	0
11	4	2	1	1	1	0
17	3	3	1	1	1	0
18	3	1	1	1	1	0
23	2	1	1	1	0	1
24	2	2	1	1	1	0
105	2	2	1	1	1	0
106	3	1	1	1	1	0
107	3	2	1	1	1	0
108	3	1	1	1	1	0
113	3	1	1	1	1	0
114	3	3	3	1	1	0
116	4	2	2	1	1	0
117	4	1	1	1	1	0
118	2	1	1	1	1	0
121	2	2	2	1	1	0
122	3	2	1	1	1	0
123	3	1	1	1	1	0
133	4	4	4	2	2	0
134	3	2	1	1	1	0
135	2	2	1	1	1	0
137	2	2	2	1	1	0
142	3	3	2	1	0	1
144	3	3	1	1	1	0
145	3	3	2	2	2	0
146	4	4	3	2	2	0
147	2	1	1	1	1	0
149	3	3	3	2	2	0
150	3	3	3	2	1	1
Totals..	88	64	49	38	34	4

medium. (Fig. 5, F.) The transfers were made by means of a sterile spatula made from a piece of a safety-razor blade fused into a glass rod.

Behavior of pedigreed single-spore strains. During the present investigation a total of 225 basidiospores were isolated from 150 individual basidia by the method described above. Of this number, 123 germinated and 86 continued to grow.

The behavior of the single-spore cultures isolated from 31 basidia of Rhizoctonia R-40 is shown in table 10, from which it will be seen that nearly all spores isolated from individual basidia were fertile irrespective of origin. The single spores isolated from 2-, 3-, or 4-spore basidia behaved

TABLE 11.—*Behavior of the second single-spore progeny obtained from one of the first single-spore cultures ($B6_2$) of R-40. (C. praticola)*

Basidium No.	Number of spores				Behavior	
	Formed	Isolated	Germinated	Grew	Fertile	Sterile
40	2	1	1	1	1	0
41	3	2	1	1	1	0
43	4	1	1	1	1	0
48	2	2	1	1	1	0
49	4	1	1	1	1	0
51	2	2	2	2	2	0
52	4	2	1	1	1	0
53	2	2	2	2	2	0
54	3	1	1	1	1	0
59	3	2	2	2	2	0
62	3	2	2	2	2	0
66	3	1	1	1	1	0
68	3	1	1	1	1	0
72	3	1	1	1	1	0
73	3	1	1	1	1	0
75	4	1	1	1	1	0
76	3	1	1	1	1	0
78	4	1	1	1	1	0
79	4	2	1	1	1	0
80	2	2	2	2	2	0
81	4	2	2	2	2	0
83	3	2	1	1	1	0
84	3	3	1	1	1	0
86	3	1	1	1	1	0
89	4	2	1	1	1	0
90	3	2	2	1	1	0
91	4	2	2	2	2	0
92	3	1	1	1	1	0
Totals	88	44	36	35	35	0

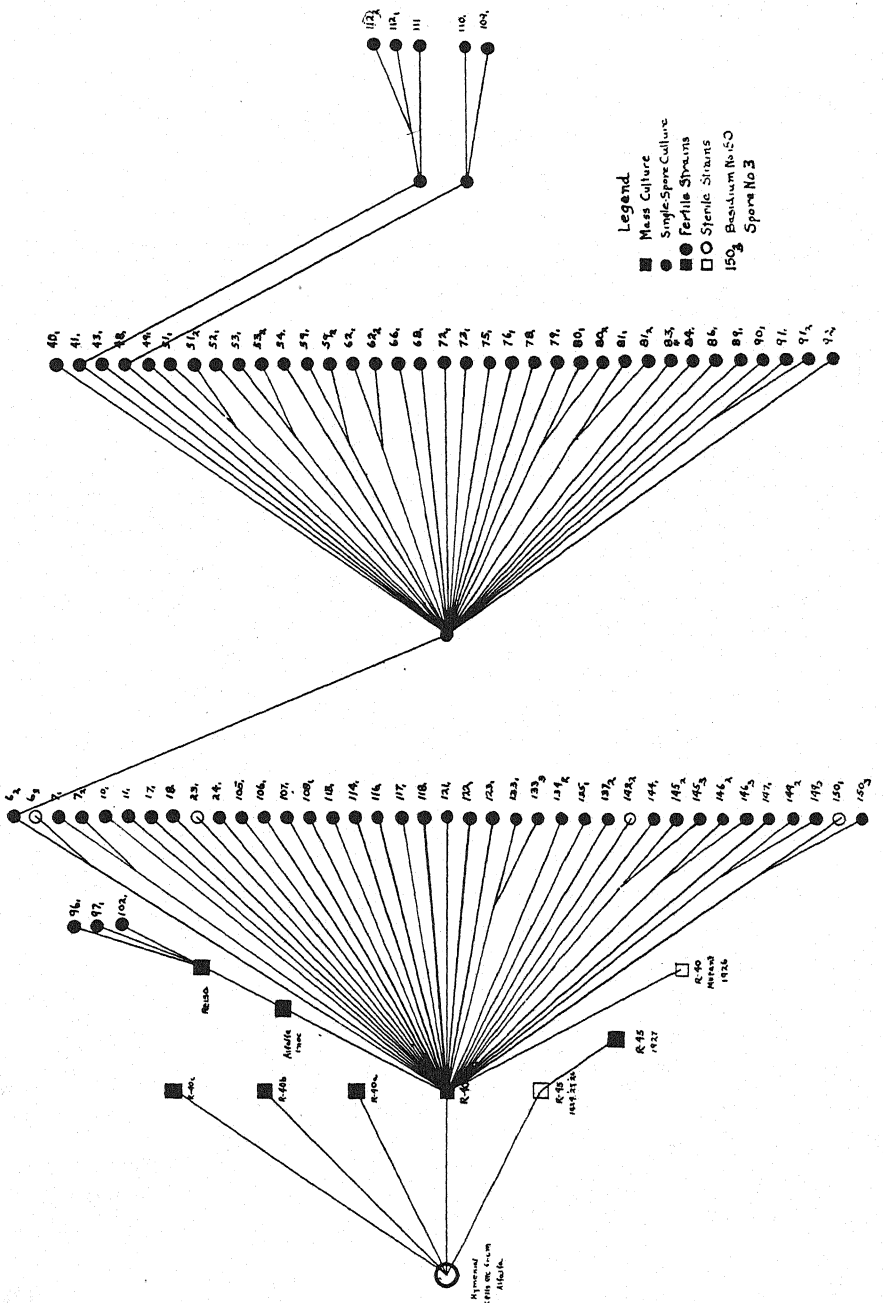


FIG. 4. Graph showing behavior of the spore-forming Rhizoctonia and its single-spore progeny.

alike. *Note, however, that four single-spore cultures were sterile and have remained so.* All attempts to stimulate basidiospore formation, by growing these cultures together in all possible combinations and in various other ways known to favor reproduction, have resulted in failure.

In order to determine whether the progenies of these spore-forming, single-spore cultures would behave in the same way as the parent culture, one fertile single-spore culture $B6_2$ (basidium No. 6, spore No. 2) was selected and grown in petri dishes as described above. When basidia and spores were abundant single spores were picked from 54 individual basidia of the latter culture. The spores from 28 basidia germinated and continued to grow. The behavior of these single-spore cultures is shown in table 11.

It is seen from the data in table 11, that the single-spore culture $B6_2$ has greater stability than the parent culture (R-40). All of the single-spore progeny of this fertile culture were found to be fertile.

The study was carried one step farther and single basidiospores were isolated from two single-spore cultures of the second series, viz, $B6_2$. Three single-spore cultures were obtained from two basidia of $B41_1$ and two from two basidia of $B48_1$. All of these formed basidiospores as abundantly as the parent and grandparent cultures.

The behavior of the spore-forming R-40 and its progeny is shown graphically in figure 4 and indicates that the fungus under consideration is homothallic. The assumption that the presence of a common sex factor is responsible for the negative results obtained in the matching of sterile forms is not sustained. The apparent sterility in these forms must be due to some other unknown cause.

THEORETICAL POSSIBILITIES INVOLVED IN THE PRESENT STUDY OF THE FORM R-40 OF C. PRATICOLA

Viability of basidiospores. It was observed during the progress of the present study that many of the basidiospores isolated by the glass-needle method failed to germinate. *It was further observed that after some spores had germinated the germ tube failed to continue to grow.* Of the total of 225 basidiospores isolated, 54.6 per cent germinated but only 36.4 per cent of the total number picked continued to grow. The failure of some basidiospores to germinate and of others to continue growth after germination opens up the question of the underlying causes.

Conditions involved. The question of technique might be raised, and it is very probable that the low percentage of germination obtained in the early isolations made from form R-40 was due largely to faulty technique. However, after the technique had been mastered sufficiently so that complete sets of basidiospores could be isolated very quickly—the entire opera-

tion of picking and planting the spores from a three-spore basidium requiring not more than five minutes—the percentage of germination had improved only 23.8 per cent. It would seem unlikely, therefore, that faulty technique was entirely responsible for the behavior of the basidiospores and especially of those failing to continue growth after germination. A number of typical cases, in which complete sets of basidiospores were isolated and in which one or more of them failed to continue to grow after germination, are shown in table 12.

TABLE 12.—*Behavior of complete sets of basidiospores isolated from individual basidia of R-40. (C. praticola)*

Basidium No.	Number of basidiospores			
	Formed	Isolated	Germinated	Grew
105	2	2	1	1
114	3	3	3	1
119	2	2	2	0
121	2	2	2	1
127	2	2	2	0
128	3	3	1	1
129	3	3	1	1
131	2	2	2	0
133	4	4	4	2
135	2	2	1	1
137	2	2	2	1
142	3	3	2	1
143	3	3	2	2
144	3	3	1	1
145	3	3	1	1
146	4	4	3	2
148	3	3	1	0
149	3	3	3	2
150	3	3	3	2

In many of the cases shown in the above table basidiospores no. 2 and no. 3 continued to grow and no. 1 failed to do so. Basidiospore no. 1 represents the last spore picked from a given basidium and the first one planted after isolation. This would indicate that if the method were faulty, this basidiospore should have had considerable advantage over the others and should have been the one to germinate and continue to grow. Müller (59) studied the viability of the basidiospores of *Corticium vagum* which he had under observation and found that they did not remain viable beyond 44 days. It is presumed that the spores remained viable for that length of time and, if so, it would seem highly improbable that the basidiospores of the apparently closely related Corticium herein considered had sufficient

time to lose their viability through drying out during the few minutes elapsing between the isolation and planting of the spores.

That variations in the medium used and on which the spores were transplanted are not a factor is shown by the details of procedure. All of the basidiospores from a given basidium were planted in marked areas not more than 1 cm. distant from each other in the same petri dish and all of the basidiospores isolated from three other basidia were planted in similarly marked areas in the same dish, all of the spores being planted within a radius of 2½ cm. of the center of the dish. (Fig. 3.) It would seem that the external conditions were alike for all spores. Yet, under these conditions, some of the basidiospores germinated and grew rapidly while others, when examined under the microscope 24 hours after isolation, were found to have germinated and to have made very feeble growth. In some cases germ tubes were formed which were from 6 to 12 microns long. Daily observations made on some of these germinated basidiospores for a period of one week failed to reveal any further growth.

The improbability that external conditions were responsible for the failure of some basidiospores to grow having been pointed out, the probable effect of other conditions should be considered.

What effect has maturity of basidiospores on germination and growth? Experience derived from continuous isolation of basidiospores of all sizes, and consequently in all stages of maturity, from hundreds of basidia, has shown that a spore at the proper stage of maturity is not difficult to isolate by the needle method herein described. With the slightest touch of the needle the mature basidiospore can be dislodged from its sterigma; whereas, it is impossible to remove an immature spore by this method. In two cases it was observed that basidiospores were actually released from their sterigmata and had attached themselves to the needle before the needle had come in contact with the spores.[14] The immature basidiospore, after contact with the needle, adheres to it so strongly that when the needle is drawn away the sterigma is bent until the force of cohesion overcomes the force of adhesion and the sterigma with its spore flies back to its original position on the basidium. The process can be repeated until the sterigma,

[14] Although violent discharge of spores, as described by Buller (14) for other Basidiomycetes, was not observed except in these two cases, there is no question in the mind of the writer but that this phenomenon takes place in connection with the liberation of the basidiospores of the fungus under investigation. Many times making single-spore isolations it was observed that plates containing great numbers of basidiospores when the cover plate had been removed, had, a short time later, very few that could be removed from their respective sterigmata. A great many sterigmata from which spores had been released could be seen about the plate, and many of the basidiospores were observed on the surface of the agar not far removed from the basidia, or on adjacent aerial hyphae of the fungus. The difficulty experienced during the early part of the work from a too rapid discharge of basidiospores was overcome somewhat by increasing the humidity of the atmosphere surrounding the isolation apparatus.

with its basidiospore still attached, is so badly injured that it collapses and is drawn in amongst the surrounding basidia and hymenial cells. It would seem, therefore, that a basidiospore which can be removed from its sterigma by the slightest contact with the needle and which is planted within two minutes after its isolation is sufficiently mature to germinate and to continue to grow.

Lethal factors have been found associated with many animals and plants. These factors have been reported to occur in yellow mice by Cuènot (24) whose work was confirmed by Little (52). Morgan (56) and his students have reported 25 or more lethal factors in *Drosophila melanogaster* Meig. In the plant kingdom these factors were postulated by de Vries and have been shown to occur in corn by Lindstrom (51), in snapdragon by Bauer (4), and in Oenothera by Shull (76), and other investigators. The lethal factors in corn are expressed at various stages during the life of the individual, as, for example, in defective seeds Jones (38), in white seedlings Emerson (31), Lindstrom (51), Demerec (26), etc. It has not been possible to demonstrate conclusively that such factors occur in fungi, and while they may not be responsible for the germination failure of some of the basidiospores of the Corticium under investigation or of growth failure of others after germination, and of the inability of still others to produce spores after apparently normal growth, yet the wide use of such theoretical factors in explaining certain phenomena must be acknowledged.

Heterothallism. It might be assumed that the reason for the nonspore-forming character of some single-spore isolations lies in the fact that the fungus is heterothallic and the spores, having a haploid consituation, do not form basidiospores. The results obtained from the preliminary matching experiments seemed to indicate such to be the case. Yet the failure to confirm these results in a clear-cut manner after repeated experimental efforts seems to indicate that basidiospore formation in this fungus is not the result of the union of sexual strains.

Further grounds for this belief are afforded from the negative results obtained from matching nonspore-forming single-spore cultures of unknown ancestry as well as pedigreed cultures isolated by the glass-needle method. These cultures were matched in all possible combinations under what are believed to be optimum conditions, but in no case were basidiospores observed to have been formed.

It might be argued that the point is still to be proved on account of the small number (4) of pedigreed nonspore-forming cultures which were matched. However, the very rare occurrence of these infertile forms would seem strong evidence to indicate that infertility is the exception rather than the rule in the Corticium under consideration.

The fact that all of the single-spore cultures isolated from the fertile single-spore culture $B6_2$ formed basidiospores (Table 11) might be considered further proof that the fungus is normally homothallic. In the case of a heterothallic fungus it is assumed that all of the spores isolated from a given basidium will have a haploid constitution, and that no single-spore culture will form spores until a union occurs between its mycelium and that of the sexually opposite form. *In no case in the present investigation was more than one nonspore-forming single-spore culture isolated from a single basidium regardless of whether it bore two, three, or four basidiospores.* This is shown in table 10, and only four cases occurred out of the 31 sets from individual basidia. In two of these cases one basidiospore was infertile, while another, isolated from the same basidium, was fertile.

It seems to be more reasonable to assume that the sterile character of some of the single-spore cultures is not due to complicated sex factors, as reported by Hanna (35) for *Coprinus lagopus,* but that it is due to some factor which has suppressed the spore-forming propensity of the fungus and has permitted only vegetative growth. It has been shown that various external factors, such as quality of food, humidity, and temperature, are controlling factors in the basidiospore formation in form R-40. However, such external factors cannot be considered responsible for the failure of some of the cultures to form basidiospores because all were grown under optimum conditions for reproduction in this fungus. The possibility of internal limiting factors is advanced. From the evidence presented it may be assumed that the original culture of R-40 is in an unstable condition, since it yields both fertile and sterile cultures as compared to the single-spore culture $B6_2$ which may be considered stable, since all of its progeny have formed spores. Just as $B6_2$ may be considered stable for spore formation, so the nonspore-forming cultures may be considered stable for nonspore-formation. Whether a single-spore culture will be fertile or sterile will depend on whether the internal factors for spore formation or for vegetative growth have the upper hand. The great variation in spore-forming capacity exhibited by the single-spore cultures of $B6_2$, some forming basidiospores abundantly, while others, scarcely any at all, seems to show that there are some grounds for the hypothesis that unknown internal factors govern basidiospore formation in the fungus studied. Whether a cytological study of nuclear behavior in the basidium will supply the answer remains to be determined.

Mutations. Mutations,[15] saltations, or aberrant forms in fungi have been reported by Blakeslee (7) in Mucor, Christensen (21), and Stevens

[15] The term "mutation" is here used not in the de Vriesian sense but rather loosely as referring to a sudden change from the parent form not necessarily so stable as to segregate out according to Mendelian principles.

(78) in Helminthosporium, Bonar (8) in Brachysporium, by Leonian (50) in Phytophthora, and by Caldis and Coons (19) in Cladosporium. These mutations found expression in various ways, such as reduced growth, lack of conidia, loss of color, pathogenicity, etc. In fact, it appears that something was lost in all of these aberrant forms. When considered from this viewpoint, the apparently abnormal sterile cultures may be considered as mutations from the normal spore-forming character of the fungus, having lost the faculty of forming basidiospores.

A mutation characterized by loss of the power of forming basidiospores seems to have occurred in a subculture made from the original spore-forming Rhizoctonia R-40. At no time had subcultures from this source failed to form basidiospores when grown under favorable conditions. During the spring and summer of 1926 in the subculture, from which the aberrant culture arose, basidiospores had formed normally and had been used as a source of inoculum for petri-dish cultures used for single-spore isolation work. After July of that year no further transfers were made until September 20, when work was resumed. Transfers made at that time yielded only mycelial and sclerotial growth. Numerous transfers made at short intervals in an attempt to reinvigorate the fungus failed to restore its original spore-forming condition. The Klebsian method of growing organisms, first in media rich in food materials to obtain abundant vegetative growth and then transferring them to media poor in food content to induce reproduction, was tried. The aberrant culture was grown in flasks of malt-extract broth and after a mat of mycelium had formed over the surface of the medium, portions of it were transferred to test tubes containing a sterile filter paper saturated with sterile distilled water. Two weeks later a microscopic examination showed no basidiospores. The checks used in these trials, a transfer from an old subculture of the fungus made nine months earlier, and a transfer from the spore-forming single-spore culture $B6_2$, formed basidiospores abundantly.

That reversibility of mutating forms of fungi is possible has been pointed out by Blakeslee (7), Leonian (50), and by Caldis and Coons (19). That the aberrant form of R-40 has not entirely lost the function of spore formation and that it may possibly revert to its former condition are shown by the fact that when grown on agar containing either maltose or malt extract, as sole source of food or stimulants, the culture did form a few scattered groups of hymenial cells and basidia. These were found by means of the microscope amongst the masses of sclerotial cells on the top plate of the petri-dish culture.

The behavior of this aberrant culture shows that some internal factor has swung the balance from its normal spore-forming condition to that which encourages only mycelial and sclerotial development. The neutrali-

zation or elimination of this factor would probably permit the return of the fungus to its original condition.

The abnormal culture referred to above is strikingly similar in behavior to the hitherto sterile culture, R-43, isolated from the original mass of hymenial material in 1924. This culture, which was observed to have formed basidiospores during 1924, 1925, and 1926 *only when grown in association with other sterile cultures* and then in only a few instances, was discovered in March, 1927, to have formed a few basidia and spores when grown alone. The previous growth on malt extract and subsequent transfer to Coons' synthetic medium in a petri dish are thought to have either stimulated the latent spore-forming factor of the fungus or disturbed an inhibiting factor, so that a few basidiospores resulted. The latter were discovered while making a critical microscopic examination of the culture. It is believed that by growing this culture in association with other infertile cultures a similar stimulation occurred which resulted in the formation of a few basidiospores. This reaction is not considered to be of a genetic nature.

DISCUSSION

I

A Rhizoctonia has been isolated which appears to be vastly different from the forms usually observed. Whereas, the common experience has been that known forms of Rhizoctonia remain sterile in culture, this new form is fertile and forms hymenial cells, basidia, and spores abundantly when grown under certain environmental conditions. It is to be called **Corticium praticola,** sp. nov.

The vegetative characters of *C. praticola* and of *C. vagum* B. & C., as obtained in culture, are similar in many respects. The mycelium is characterized by a uniform diameter, smooth wall, and constrictions where branching occurs. Further, as described by Duggar and Stewart (29) for *C. vagum,* a septum is present very near the constriction. Anastomoses are abundant. The sclerotia, while not so large nor so numerous as those of *C. vagum,* are similar to those of the latter both as young cells and as fully developed sclerotia. (Fig. 5, B.) These similarities indicate that the fungus under consideration, in its vegetative stage, is unquestionably a Rhizoctonia.

Although many similarities exist in the vegetative growth of *Corticium praticola* and that of *C. vagum,* some differences are apparent. The most striking one is the hyaline or white character of the mycelium of the new species as contrasted with the brown color associated with *C. vagum.* Specific morphological differences occur in the perfect stages of the two species, such as differences in size of basidiospores as well as the number of spores

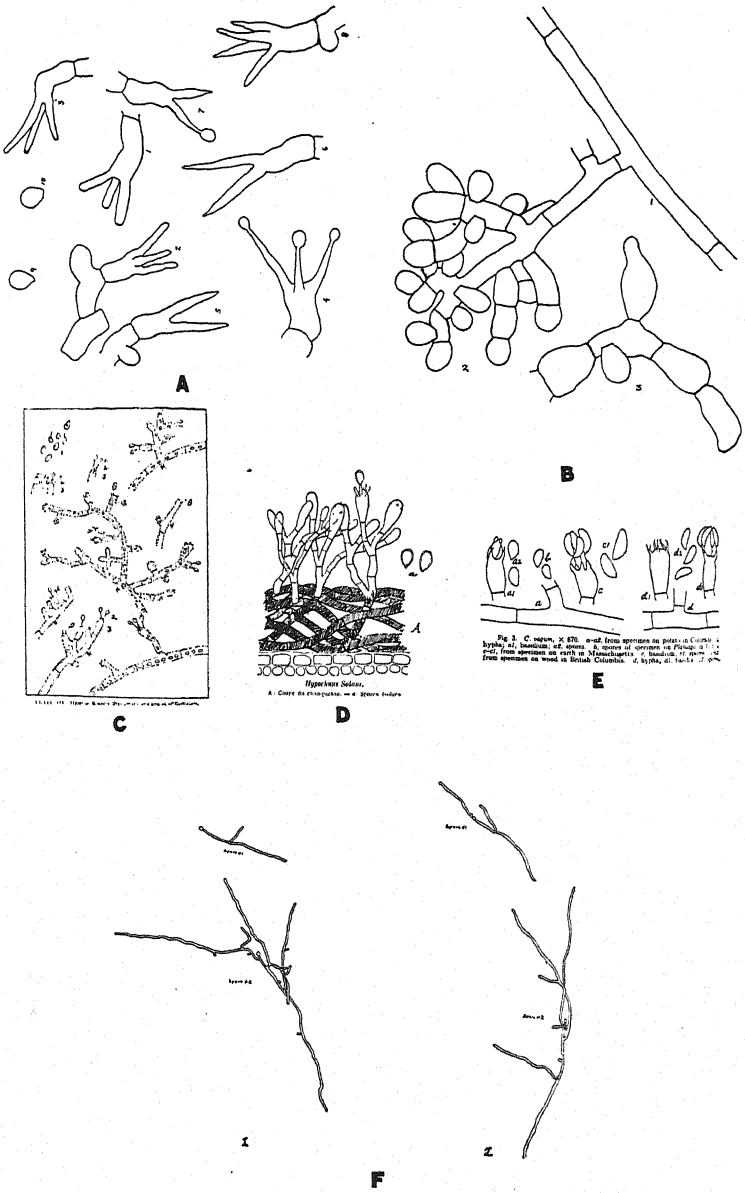


FIG. 5. A. Basidia of *Corticium praticola* on artificial media. Camera lucida drawings, Bausch & Lomb objective 3 mm., ocular 10.

Figs. 1–4. Immature basidia. Figs. 5–8. Mature basidia, spores discharged. Figs. 9–10. Mature basidiospores.

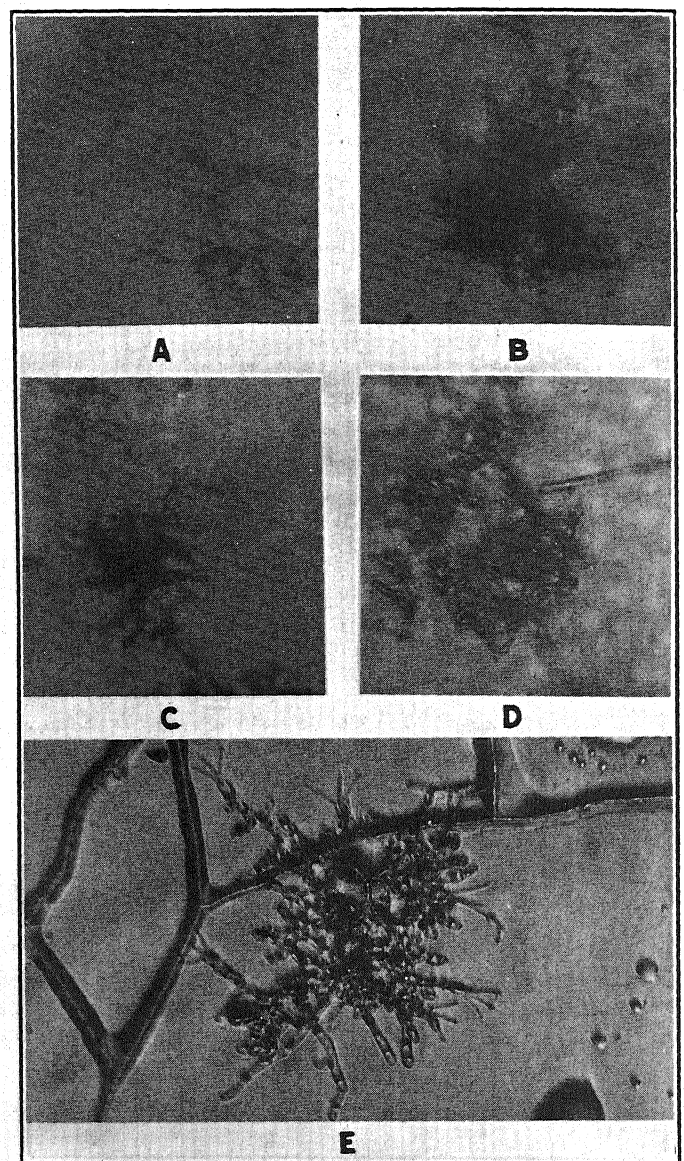
B. *C. praticola* on artificial medium. Camera lucida drawings.

Fig. 1. Vegetative hypha. Fig. 2. Hymenial cells. Fig. 3. Sclerotial cells.

C. *Corticium vagum* B. & C. from Rolfs (71). D. *Hypochnus solani* Prill. & Delacr. from Prillieux and Delacroix (64). *E. Corticium vagum* B. & C. from Burt (17).

F. Isolation of single spores.

Fig. 1. Two spores isolated from basidium No. 62, showing difference in rate of growth. Camera lucida drawing made 48 hours after isolation of basidiospores. B. & L. objective 16 mm., ocular 10.



Photomicrographs of *C. praticola*. (Enlarged.)

FIG. 6. A. Basidium, near surface of medium in petri dish; two basidiospores.
B. Basidium with three spores.
C. Basidium with four spores.
D. Isolation of single spores. Note basidiospore attached to side of needle adjacent to sterigma from which it was removed.
E. *C. praticola* on under surface of top plate of petri dish, showing prevalence of basidia bearing three basidiospores.

formed by each basidium and differences in lengths of sterigmata. According to Burt (18) the number of basidiospores formed on each basidium of *Corticium vagum* (*R. solani* Kühn) varies from 4 to 6, while in *C. praticola* the basidiospores vary in number from 1 to 4 on each basidium, three being the number most frequently observed. (Fig. 6, E.) The smaller spore size and the unusual length of the sterigmata of *C. praticola* are also thought to be significant.

It should be pointed out that these comparisons are made between the perfect stages of two fungi, one of which developed on artificial media, the other under natural conditions on natural hosts. It is perhaps unfortunate that the cultures of *C. vagum* to which *C. praticola* was compared in its vegetative stages did not form the perfect stage in culture so that a comparison of basidia of exactly similar origin could be made between the two forms. However, Müller (59) found that the perfect stage of *C. vagum* (*H. solani*) which he obtained under artificial conditions was not strikingly different from descriptions given in the literature. It would seem unlikely, therefore, that the perfect stage of *C. vagum* on artificial media would be much different than on its natural hosts. No differences of basidial form or structure attributable to place of origin were found in *Corticium praticola*. When cultured on alfalfa seedlings kept under very humid conditions, the perfect stage was obtained. Microscopic examination and measurement of the hymenial cells, basidia, sterigmata, and basidiospores showed them to be the same as similar structures of the fungus formed on artificial media.

II

The present study has shown the important rôle played by external factors during reproduction in *C. praticola* and has demonstrated that in the environmental complex there are certain factors on the presence of which reproduction is dependent. It has shown further that these factors must be present in certain intensities for optimum reproduction.

Humidity, temperature, and aeration were found to be controlling factors. Light appeared to encourage spore formation, whereas reaction of substratum, in the intensities tried, was found to have no effect upon either growth or reproduction.

Of all of the contributing external factors, that of humidity, influencing probably the transpiration of the fungus, seemed to be most important. This was the controlling factor when all other conditions were favorable for reproduction. Too low humidity prevented basidiospore formation; whereas, too great an intensity retarded the phenomenon. Optimum basidiospore formation occurred at approximately 60 per cent humidity. It is noteworthy that the occurrence of *Corticium vagum* in nature has also been correlated with abundant moisture.

Temperature also was found to be a controlling factor. Although basidiospore formation occurred over a considerable range of temperature (16.0° to 24.3° C.), the optimum appeared to be about 20.0° C. Vegetation was found to have a wider range than reproduction. Similar results were reported by Klebs (43), Coons (22), and others. The temperature studies showed that sclerotium formation and that of basidiospores are opposed to each other. The optimum temperature for the former was very close to the inhibiting point for the latter.

Oxygen was found necessary for the growth as well as for reproduction of the fungus concerned. Aeration of cultures was found to encourage basidiospore formation, while too great an oxygen tension was inhibitive.

Light was not found to be an essential factor in reproduction but seemed to favor it. Whether the increase in the number of basidiospores formed in the light was due to an oxidation response, as reported by Coons (22), was not determined. The studies on the effect of light gave further proof that sclerotium and basidiospore formation are opposed to each other. Sclerotia were formed only in those cultures which were kept in the dark. In other experiments it was found that conditions unfavorable to the formation of basidiospores usually were favorable for the formation of sclerotia.

The reaction of the substratum, a controlling factor for the growth and reproduction of many organisms, was found to have no effect on basidiospore formation of the Corticium under consideration.

III

Just as certain external factors were found to be controlling factors in basidiospore formation, so too it seems that internal or genetic factors exert a similar influence.

It has been shown that some single-spore isolation of *Corticium praticola* remained sterile when grown under conditions which resulted in abundant formation of basidiospores in others. The failure to form spores is thought to be due to some inherited factor which does not respond to the stimuli exerted by the external complex.

Sterility in organisms has been shown in many cases to be due to the segregation of sex factors. Some grounds for the belief that heterothallism existed in the fungus under consideration were obtained in the early part of the investigation. Basidiospores were obtained when two previously sterile cultures were grown together. A few confirmatory results also were obtained, adding more weight to the supposition. However, the spore formation in these cases was always meager and not abundant as was the case in the fertile cultures. By a study of single-spore cultures obtained from individual basidia of a fertile form the true nature of the fungus was learned. In the absence of heterothallism, it is concluded that the fungus

is homothallic, since all of the pedigreed single-spore forms, with only four exceptions, have formed basidiospores. All of these fertile cultures behaved alike irrespective of whether they had their origin on a 2-, 3-, or 4-spore basidium.

That the sterility of some of the pedigreed single-spore forms, as well as those of unknown origin, is not due to a haploid genetic constitution is indicated by their failure to form basidiospores when grown together in all possible combinations. The fact that all of the progeny of one single-spore isolation ($B6_2$) were fertile indicates that the situation here is apparently not analogous to that found in heterothallic forms and that some internal factor other than sex is responsible for the sterility of the cultures referred to above.

The presence of some factor in *Corticium praticola* which affects spore formation is shown by the behavior of the progeny of the single-spore isolation $B6_2$ referred to in the foregoing. Some of these cultures formed basidiospores very abundantly, while others, grown under similar environment, formed scarcely any at all. Further proof of the existence of such controlling internal factors was obtained from the abnormal culture which reverted from the spore-forming to sterile condition. The function of spore formation is not lost in this aberrant culture but is merely suppressed, as was shown by causing it to form a few basidiospores when grown under certain conditions.

It is believed that similar internal factors are operating in the case of the apparently sterile form R-43, isolated from the original mass of hymenial material in 1924. These factors were sufficiently overcome in the spring of 1927 so that the culture formed a few basidiospores when grown alone. It is thought that growing the apparently sterile form in association with other infertile cultures had a similar stimulative effect on the internal condition of this fungus.

SUMMARY

1. The "Rhizoctonia problem" has been approached from an entirely new angle: (a) by the discovery of a new species which was more easily subjected to study than the hitherto known forms, (b) by the application of the more modern technique in the determination of its biological characteristics.

2. This Rhizoctonia forms the perfect stage, which shows that it belongs to the Basidiomycetes and that it is a Corticium.

3. The hymenial stage has been regularly developed in culture on artificial media and on inoculated alfalfa plants. From the latter the fungus was reisolated and found to have retained its ability to produce basidiospores.

4. The effect of external factors of the environment on the formation of the hymenium and basidiospores has been determined. It is found that humidity, oxygen, and temperature are controlling factors. Light, although a contributing factor for optimum basidiospore formation, is to be considered of secondary value. The reaction of the medium had no influence under the conditions to which the cultures were subjected. The most favorable food supply consisted in a medium composed of malt extract and maltose supplemented with potassium dihydrogen phosphate, calcium nitrate, and magnesium sulphate.

5. An analysis by means of single-spore cultures, to determine the sexual conditions present in each of the basidiospores of any one basidium, has convinced the author that this Corticium is homothallic.

6. The discovery of a sterile form of this species, derived from the same source as several fertile ones, led to a detailed study of single-spore generations of both the fertile and sterile forms, to determine whether the causes were genetic or due to unfavorable conditions.

7. The sterile condition of the form which did not produce basidiospores was found to be unaffected by varying the external factors, and it was concluded that such cultures arose from internal disturbances in the mycelium.

8. An analysis of the sterile versus the fertile forms also showed that the sterility was not associated with sex factors.

9. The new plant is considered to belong to the genus Corticium and the name **Corticium praticola**, sp. nov. is proposed for it.

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EXPERIMENTS WITH BLUESTAIN FUNGI IN SOUTHERN PINES

RALPH M. NELSON AND J. A. BEAL

ASSOCIATION OF BLUESTAIN WITH BARK BEETLES

The close association between bluestain and bark beetles in living trees has long been known. Von Schrenk (6) described the death of a large number of western yellow pines, *Pinus ponderosa* Laws., in the Black Hills and stated that wood of trees attacked by the Black Hills beetle, *Dendroctonus ponderosae* Hopk., became stained within a few weeks by a species of Ceratostomella. Münch (5) and MacCallum (4) also have reported the occurrence of bluestain in conifers attacked by bark beetles. Craighead (2) has mentioned their invariable association with Dendroctonus attack and has suggested that the fungi play an important rôle in the death of beetle-attacked trees. It was largely through his stimulating interest that this joint study was begun.

Bluestain in southern pines, except in association with beetle attack, is uncommon, although it is occasionally found in pines which have been severely wounded, such as in dry faces of turpentined trees. In Sweden (3) spruce trees which have been blazed and left standing for several years are frequently reported stained. Colley and Person (1) have found that of 234 zones of bluestain in western yellow pines only 4 per cent appeared not to have originated from entrance holes of the western pine beetle, *Dendroctonus brevicomis* Lec. Of 218 zones of bluestain in shortleaf pine, *Pinus echinata* Mill., attacked by the southern pine beetle, *Dendroctonus frontalis* Zimm., the writers found 97 per cent directly associated with the entrance holes of the beetle.

The circumstantial evidence presented above suggests that the bark beetles carry the bluestain fungi into their tunnels and thereby inoculate the trees.

INSECTS ASSOCIATED WITH BLUESTAIN

The following discussion deals briefly with the life history and habits of the bark beetles included in this preliminary study.

The southern pine beetle is the most destructive insect enemy of pines in the Southern States. It attacks pines of all the species that occur within its range. The adult insect is a small, brownish or blackish beetle, about one-eighth inch long. It attacks the central and upper portions of healthy pine trees, weakened individuals, and occasionally freshly felled logs. The beetles bore through the bark and excavate long serpentine galleries which may run in any direction and which extend through the inner layers of the

bark and mark the surface of the wood. Eggs are laid in small niches along the galleries and the young bore away from the galleries about one-half inch during the course of their development. The tunneling of the adults, therefore, and not that of the young, has in the past been held largely responsible for the death of the trees, because fading of the foliage occurs before the larvae are half grown.

One of the engraver beetles, *Ips calligraphus* Germ., usually considered of secondary importance, sometimes attacks healthy trees. It is usually found associated with the southern pine beetle, but confines its activities to those parts of the trees not occupied by other species of insects. The adult galleries are usually perpendicular, but often branch in many directions.

The black turpentine beetle, *Dendroctonus terebrans* Oliv., may be found on pines throughout the South. It attacks logs, stumps, and occasionally the bases of green trees. Seldom, if ever, is it able to establish itself in large numbers, because the individuals are usually "pitched out." Adult galleries extend in a vertical direction and, therefore, do not girdle the trees. This beetle, because of its habits, can inoculate only a relatively small part of the tree with bluestain. The two other beetles may attack almost any part of the boles of trees in large numbers and are, therefore, more capable agents of inoculation.

A number of other species of bark beetles are associated both with dying pines and with bluestain, but the experimental inoculations here described were made only with stain fungi isolated from trees attacked by the three above-named beetles.

METHODS

The object of the experiments here reported was to determine the ability of bluestain fungi to cause death of trees independent of beetle attack. Isolations were made from the blue wood of trees attacked by bark beetles and also from adult beetles. In a few cases bluestain fungi were obtained from adult beetles taken from newly made tunnels without a sign of stain. The preliminary study indicates that the isolated fungi consist of several species or strains of Ceratostomella or very closely related fungi. The stain fungi associated with the engraver beetles as a group, the turpentine beetles, and the southern pine beetles can be distinguished readily from each other in culture.

Pitch pines, *Pinus rigida* Mill., and shortleaf pines, *Pinus echinata* Mill., 4 to 8 inches in diameter, breast high, were selected at random on the Bent Creek Experimental Forest near Asheville, N. C., and inoculated with staining fungi isolated from blued wood of trees attacked respectively by *Dendroctonus frontalis*, *D. terebrans*, and *Ips calligraphus*.

The three types of organisms were grown in Kolle flasks on rice which had been boiled to a mid-soft consistency and then autoclaved for 20 minutes at 15 pounds pressure. With this material each tree in the first group (Table 1) was inoculated with 5 to 9 poultices arranged obliquely one above

TABLE 1.—*Results of inoculating southern pines with fungi isolated from bluestained wood of trees attacked by bark beetles*

Culture no.	Species of pine inoculated	Method of inoculation	No. of places inoculated[1]	Sources of inoculum; from tree attacked by	Weeks after inoculation	Condition of tree
12	*Pinus rigida*	Poultice	5	*D. terebrans*	8	Dead
	do.	do.	5	Check (sterile rice)	20	Live
6	do.	do.	5	*I. calligraphus*	20	do.
6	*P. echinata*	do.	5	do.	20	do.
14	do.	do.	9	*D. frontalis*	10	Dying
14	do.	do.	9	do.	18	Live
6	do.	do.	7	*I. calligraphus*	10	Dying
6	do.	do.	7	do.	10	Dead
12	do.	do.	9	*D. terebrans*	10	do.
32	do.	do.	9	*D. frontalis*	13	do.
32	do.	do.[2]	9	do.	5	do.
32	do.	do.[2]	9	do.	8	do.
14	*P. rigida*	Drill holes	200	*D. frontalis*	21	Live
	do.	do.	200	Check	21	do.
6	*P. echinata*	do.	250	*I. calligraphus*	21	do.
	do.	do.	250	Check	21	do.
14	do.	do.	200	*D. frontalis*	20	do.
11	*P. echinata*	Grease gun	100	*D. frontalis*	14	Dead
11	do.	do.	80	do.	14	do.
11	do.	do.	100	do.	10	do.
11	do.	do.	80	do.	14	do.
11	do.	do.	80	do.	15	do.
11	do.	do.	90	do.	14	Live
11	do.	do.	80	Check	13	do.
11	*P. rigida*	do.	90	*D. frontalis*	13	Dead
32	do.	do.	60	do.	13	do.

[1] Number of grease-gun and drill-hole inoculations approximate.

[2] Poulticed every 2 feet for 18 feet. Each poultice girdled the tree.

the other in an ascending spiral, in such manner as to girdle the tree at least once in approximately a 6-foot length of bole. The bark, in rectangular patches, about 3 by 5 inches in size, was cut through to the wood and removed, a rice-fungus poultice applied to the exposed surface and the bark patches fastened with nails. The second group of trees was inoculated through small drill holes bored through the bark and usually not more than one-half inch into the sapwood. The 200 to 250 holes in each tree were spaced about 4 inches apart, both vertically and horizontally, and were dis-

tributed over a 15- to 20-foot length of bole. The inoculum for this second group, grown on malt-extract agar, was introduced into the holes with a small piece of wood. In the third group the organism, grown on rice, was forcibly introduced tangentially into the cambium region by means of an automobile grease gun fitted with a slender copper point. The result was a tangential elliptical pocket usually not more than one square inch in area. From 60 to 100 inoculations were made in each tree, encircling the bole, and extending upwards about 15 feet. In general, this is the part of the bole attacked by *Dendroctonus frontalis.*

EXPERIMENTAL RESULTS

So far as has been observed, there has been a heavy staining of the wood beneath each poultice and beneath almost every grease-gun inoculation. Grease-gun inoculations, from which the stain did not spread, were generally on the lower part of the bole. Here the moisture content of the wood may have been sufficiently high to inhibit growth of the organism. A large number of the points inoculated through drill holes remained unstained, although the percentage has not been determined.

The stain spreads from the point of inoculation chiefly in a radial and longitudinal direction. Beneath poultices it may penetrate an inch or more radially and several inches longitudinally in a week's time. It appears that if inoculations are so made that all the strands of water-conducting elements pass through stained zones at some point the tree will eventually succumb. The vertical extent of the stained portions probably influences the rapidity of death of the inoculated tree.

In the poulticed trees such areas were confined to a rather limited longitudinal distance. Usually a length of not more than 5 or 6 feet of the bole had been inoculated. In the trees inoculated through drill holes a comparatively large volume of the wood remained uninfected because the tangential spread of stain from the straight vertical series of holes was slight. For this reason much stoppage of conduction was not expected. At the time of last examination, the three trees inoculated through drill holes and the two check trees with drill holes appeared to be normal. A much greater amount of inoculum was used in the grease-gun method and, because the 60 to 100 points of inoculation were scattered over a 15-foot length of bole, and favored tangential extension of stain, much stain resulted. In some of the dead trees examined, the entire sapwood was heavily stained for a distance of 10 or 12 feet. The single tree which did not die produced a great quantity of resin at each point of inoculation and only approximately 35 per cent of the volume of sapwood in the region of inoculation was stained.

On beetle-attacked and in inoculated trees mycelium of bluestain fungi grows more abundantly in the medullary rays than in other tissues, although it is by no means confined entirely to the rays. Hyphae are frequently found growing within the lumina of tracheids. Microscopic examination of stained wood shows that the hyphae frequently break down the end walls of the ray parenchyma cells. These cells are usually considered to be the only cells in the xylem which remain alive for a long time after formation and some investigators believe that they have some influence on the conduction of water in living trees. The death of the ray parenchyma through the action of the bluestain fungi may conceivably interfere with the conduction of water.

Bluestained wood in beetle-attacked trees has been ordinarily found by the writers to contain less moisture than unstained portions in the same cross section. The moisture content of trees attacked by bark beetles apparently influences the development of the brood and also influences the growth of the bluestaining fungi. An examination of the wood of fire-scorched trees, subsequently attacked by the southern pine beetle, showed that the moisture content of the wood was unusually high. It also revealed a much retarded development of bluestain fungi and a decided check of development of the beetle broods. Bluestain in wood appears to influence the rate of water absorption, for, in a preliminary test on submerged sapwood blocks of shortleaf pine, unstained blocks absorbed rather uniformly an average of 33 per cent of water, based on green weight, as against 29 per cent absorbed by partly-stained blocks and 28 per cent by heavily-stained blocks. The above blocks were approximately of the same size and were cut from the same tree.

Trees in which the southern pine beetle is able to establish itself in large numbers die within a few weeks. The junior writer has severely wounded many pines in many ways. In some cases the bark and cambium have been removed for a distance of several feet; in other cases trees have been girdled by numerous saw cuts or by deep chipping. It has been observed that trees in which the southern pine beetle and accompanying stain fungi are able to establish themselves always die more quickly than mechanically-injured trees.

CONCLUSIONS AND SUMMARY

The writers are of the opinion that the girdling of pines by the tunnels of the southern pine beetles is insufficient to account for the rapid death of attacked trees. The preliminary experiments show that bluestain fungi, together with the wounding which accompanies inoculation, may kill pines in a comparatively short time. It is, therefore, suggested that bluestain organisms may play an important rôle in the death of pines attacked by

the southern pine beetles. Further inoculations with less severe wounding and more numerous controls will be made by the writers.

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A NEW SCLEROSPORA FROM AUSTRALIA

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In the case of the several known species of Sclerospora such practical questions as their injury to various valuable gramineous crops and their immediate restriction and control necessarily have been the first concern of the plant pathologist. In addition, however, the problem of the original sources of these parasites is one of some interest. In this connection the occurrence of Sclerosporas on wild hosts under natural conditions is of significance.

In February, 1928, Dr. R. J. Noble, Biologist to the Department of Agriculture of New South Wales, found at Glen Innes, clumps of *Sorghum plumosum* Beauvois, a native grass locally known as "wild sorghum," the leaves of which showed a striking fraying into tangled fibers much like that which *Sclerospora graminicola* (Sacc.) Schroet. produces in various Setarias. Since, however, the resting spores which he found among the injured leaf fibers differed somewhat in size and form from those typical of that common species of Sclerospora and since he knew the writer's interest in this genus, Dr. Noble very kindly sent the specimens to the writer for further study.

The fungus, indeed, proves to be a new species quite distinct from the related *S. graminicola* in the structure and color of the spores as well as in their consistently smaller size. Moreover, it occurs, apparently endemic, under natural conditions on a wild grass native to Australia, a species which has not been reported as host for any Sclerospora hitherto. For these reasons it is hoped that the following note may be of interest.

EFFECT ON THE HOST

In general appearance the infected plants were somewhat dried and shrivelled while the leaves, through disintegration of the tissue between their fibrovascular bundles, had shredded into tangled skeins of withered thread-like fibers (Fig. 1, A). Such symptoms are typical of advanced development of the oogonial resting-spore phase of a number of species of Sclerospora, and, as was natural, very few traces of the earlier lineate discolorations of the leaves still lingered. No evidences of a conidial or sporangial phase were seen. The mycelium, as was to be expected in an oogonial infection thus far matured, persisted as scanty, shrivelled fragments of contorted, irregular hyphae, here and there in the interstices

[1] For a grant from the Milton Fund of Harvard University which has made possible this and other comparative studies of the Sclerosporas, the writer wishes to express his gratitude.

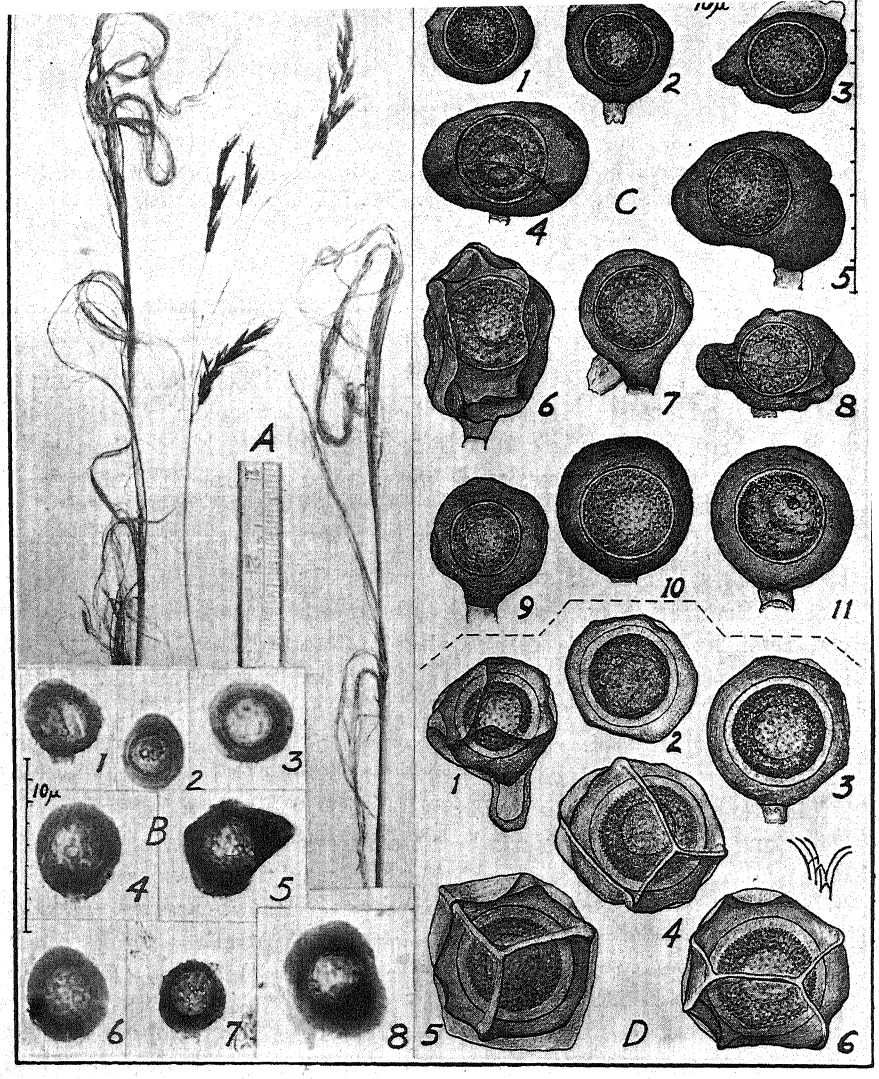


FIG. 1. A. Specimens of *Sorghum plumosum*, infected by **Sclerospora noblei** n. sp. from material collected at Glen Innes, New South Wales, in February, 1928, by Dr. R. J. Noble, showing the characteristic fraying of the infected leaves into tangled snarls of fibrovascular bundles with few fragments of disintegrated interfascicular tissue. Portion of healthy inflorescence shown in the center for contrast. Photograph, scale in inches, × ½.

B. Resting spores of **S. noblei**, showing their characteristic shape and structure and the relatively small size and thin wall of the oospore within. Photomicrographs, scale in microns, present magnification about 250 ×.

C. Resting spores of **S. noblei**; figures 1 to 4, 7, and 9 to 11 being most typically representative in their rounded shape, relatively smooth surface and thick dark oogonial wall, and in the relatively small size and thin wall of the oospore; figures 5, 6 and 8, showing the less regular forms occasionally seen. Camera lucida drawings, scale in microns, magnification about 375 ×.

D. Resting spores of *S. graminicola* var. *Andropogonis Sorghi* Kulk. from *Sorghum vulgare* from India. Figures 1 to 3 from Kirkee, India, collected by S. N. Mitra, March 10, 1905, kindly furnished by E. J. Butler, comparable to those of **S. noblei** in size, shape and configuration but distinguished by their larger and thicker walled oospores. Figures 4 to 6, from Bombay, India, collected by Dr. B. N. Uppal, December 10, 1927, and sent by him, more obviously distinct from **S. noblei** and more nearly approaching typical *S. graminicola* of Setaria in the polyhedral shape and ridged prominences of the oogonium. Drawings and magnification as in C.

between the disorganized leaf cells. In a few clumps only did Dr. Noble find cases of foliar proliferation on less heavily infected plants.

CHARACTERISTICS OF THE FUNGUS

The resting spores, which were produced in great numbers in the diseased plants, were visible, even through a hand lens, as minute, glistening, resinous-looking specks clinging among the fragments of disorganized tissue along the vascular bundles. In this, as in other species of Sclerospora, the resting spores are heavy-walled bodies comprising the spore proper, a spherical, hyaline, thick-walled oospore, surrounded by an outer coat, the closely adherent oogonial wall, usually much thickened, dark in color and decidedly modified in composition. In its shape, the resting spore, or more properly the oogonium, is usually approximately ovoid, ellipsoid, pyriform, or subspherical (Fig. 1, B 1–4, 6–8, C 1, 2, 4, 7, 9–11); occasionally somewhat angular with a few bluntly-rounded projections protruding unsymmetrically (Fig. 1, B 5, C 3); rarely very irregular, perhaps almost pyramidal, or gibbously bulging with large bulbous outgrowths (Fig. 1, C 5, 8).

The oogonial wall is typically very thick and dark. Its outer surface may be quite smooth, or it may be somewhat roughened and scabrous with irregular scabby patches. Occasionally it rises to rounded bulging protrusions (Fig. 1, C 2, 8), or even to narrow wing-like outgrowths (Fig. 1, C 3), and rarely to inconspicuous ridges, indistinctly anastomosing, but even on oogonia of very irregular shape the surface does not show the flattened, somewhat polygonal faces bounded by definite intersecting ridges that are noticeable in *Sclerospora graminicola* (*cf.* Fig. 1, D 4–6). The surface sculpturing on resting spores of Sclerospora as a whole appears to result in part from the fact that as the spore increases in size its developing wall conforms to the spaces between the surrounding host cells as they are forced apart, and also from the fact that fragments of the walls of cells thus killed become modified, darkened and confluent with the spore surface. The superficial configuration, therefore, may vary somewhat according to the tissue structure of the host leaf in which the spores develop. Hence it is possible that if this Sclerospora from *Sorghum plumosum* should infect other hosts, differences in the surface markings of the spore wall might develop in conformity to the different cellular structure of the surrounding tissue.

In color the oogonial wall varies considerably, ranging from the Mars Yellow (15 i), of Ridgways "Color Standards" to a rich brown (Brussels Brown 15 m), with a shade like that of dark amber near Sudan Brown (15 k) perhaps the most common.

The wall is typically very closely adherent to that of the single oospore within, seldom showing even small crevices between them, and never arch-

ing out into angular prominences which leave relatively large spaces around the oospore within, as is characteristic in the resting spores of some of the larger species of Sclerospora. In this species the surface irregularities, the angular, ridged, or rounded prominences, involve actual thickenings of the wall and do not result from outfolding or outbuckling of a wall of more uniform thickness. Consequently the enclosed oospore, although usually nearly central, is in some cases eccentric in position (Fig. 1, C 4–6, 10). Also it may occupy only a relatively small part of the whole body (Fig. 1, C 4, 5, 6), its diameter being perhaps $\frac{1}{2}$ or $\frac{1}{3}$ or rarely even as little as only $\frac{1}{4}$ of the greatest outside dimension of the resting spore as a whole.

Adhering to the oogonium there is frequently a portion of what had been the oogonial stalk (Fig. 1, B 2, 4, 7, C 2, 4–11), a small hyphal fragment, usually hyaline, pallid yellow, or golden, rarely as dark as the thick oogonial wall. This fragment is much more frequently present than in such species as *Sclerospora graminicola* and shows the oogonial nature of the resting spore rather clearly. This frequent occurrence leads one to believe that the oogonia had, for the most part, developed terminally on branches of the mycelium within the host tissue. Very rarely there may be seen, adhering to the oogonium, a fragment which might be interpreted as the remnant of an antheridium (Fig. 1, C 7).

The outside dimensions of the resting spores, as would be expected in bodies varying so markedly in shape, show considerable range, the diameter when approximately spherical, or the greatest dimension when irregular, varying from 28 to 44 μ (*cf.* Fig. 1, B 1–8, C 1–11). The thickness of the oogonial wall, most commonly ranging from 5 to 10 μ, at times may be as little as 3 μ, but very frequently in thickened spots is as much as 20 μ.

The oospores are almost invariably spherical in shape. Their size also is very uniform, the relatively narrow range, from 23 to 28.9 μ in diameter, including 508 of the 600 spores measured, with the size mode falling in the class (containing 230 spores) between 25 to 26.9 μ. The wall is quite smooth, of tough, homogeneous material, hyaline or with a faint tinge of golden or very pale amber color, varying somewhat in thickness but most frequently 1 to 1.5 microns, distinctly thinner than that of most other species.

The content of the oospore is hyaline or of the pallid grayish color of protoplasm, with a finely granular matrix in which occur denser granulation and clumps of granules, frequently showing at or near the center an irregular vacuolate or clear area in which, or perhaps slightly to one side, occurs a refractive, oily, slightly gold-tinged substance, either as several small globules (Fig. 1, B 1, 2), or as one large mass (Fig. 1, B 4, C 4, 6). These structural features were determined, not only from oospores freed

TABLE 1.—*Measurements of the diameters of 600 oospores of Sclerospora noblei arranged in size classes to show frequencies*

Diameter	
Classes	Number of oospores in 600
μ	
19 to 20.9	4
21 to 22.9	27
23 to 24.9	114
25 to 26.9	230
27 to 28.9	164
29 to 30.9	56
31 to 32.9	13
33 to 34.9	2

from the enclosing oogonial wall by careful manipulation, but also from those still in place in material mounted in lacto-phenol or similar clearing mixtures. The measurements given in table 1 were made, in distilled water, of oospores still *in situ,* as by proper adjustment of light it was found, on comparison with free oospores, that measuring thus was satisfactorily accurate.

Germination of the oospores was not observed.

IDENTITY OF THE FUNGUS

In connection with the identity of this fungus the only one of the known Sclerosporas that need be considered is *S. graminicola.* This species, because its general effect on the host is similar, because its resting spores approach those of the Australian in size, and because it is the only member of the genus which, in its oogonial condition, has been reported on any sorghum, naturally invites comparison. For such comparison, material of *S. graminicola* on Setaria (Chaetochloa) is presumably most typical, as on this genus of grass the species was originally described and with very few exceptions has been reported since. From such typical material of *S. graminicola* the Australian species stands out without possibility of confusion, for the size, structure and color of the resting spore (*i.e.*, oogonial) wall, and the dimensions of the oospore within, as given in detail above, distinguish it sharply from *S. graminicola* in which the resting spores are larger in outside dimensions, usually have flattened polygonal faces bounded by intersecting ridges, very rarely show any traces of an oogonial stalk, have an oogonial wall that is thinner, of paler color, more transparent, less variable in thickness, arching out here and there to leave distinct spaces around the oospore within; while the oospore itself is markedly larger, most

frequently 33 to 34.9 μ in diameter, and the wall much thicker, most frequently 2 to 4 μ, as opposed to the 25–26.9 μ diameter and 1 to 1.5 μ wall thickness of the Australian.

It is necessary, also, to compare the Australian species with material which in a few cases has been described as *S. graminicola* on cultivated sorghum (*Sorghum vulgare* Pers., *Holcus sorghum* L., *Andropogon sorghum* Brot.) in Africa (3), and in India (2, 4). From India, specimens kindly furnished by Butler, by Kulkarni, and by Uppal have been examined and found to show some differences from the typical *S. graminicola* chiefly in size of the oospores, which in some lots show the most common diameter of 37 to 38.9 μ even when the general characters of shape, size and surface of the oogonia (*cf.* Fig. 1, D 4–6) agree with typical material. That there are differences in the oogonial phase on this host is in agreement with the fact that Kulkarni (4) found in the conidial phase the very distinctive character that the conidia germinated always by germ tubes, not zoospores, and on it he established the variety *Andropogonis sorghi.* In a recent letter also Dr. B. N. Uppal reports that in his cross inoculations he finds, as did Kulkarni, that spores from sorghum will easily infect sorghum but not other hosts. All this seems still further to bear out the writer's suggestion (6, p. 781) that this variety is specifically distinct from *S. graminicola* and stimulates interest, in the case of the Australian species, in determining the characteristics of its conidial phase, if, indeed, one does develop, and in finding what may result from extensive cross inoculations to sorghum and related crops.

The specimens on sorghum from India do show some resting spores which in general shape, surface contour, and size, approximate those of the Australian species (*cf.* Fig. 1, D 1–3). Even these, however, are distinguished by the larger size of the oospores (167 out of 200 falling between 33 to 40.9 μ in diameter) and the greater thickness of their wall, which is most frequently 1.5 to 4 μ.

When all these points are considered it seems clear that this Australian species is distinct, not only from typical *S. graminicola,* but also from its variety on cultivated sorghum and must be recognized as a separate species.

Its diagnosis, therefore, is given as follows:

Sclerospora noblei. Resting spores showing rather obviously that they comprise a modified oogonium with oospore within. Oogonium usually ovoid, ellipsoid, pyriform or subspherical in shape, occasionally with bluntly rounded projections rendering it gibbous and unsymmetrical, ranging in diameter from 28 to 44 μ. Oogonial wall closely adherent to the oospore within, the exterior protrusions not representing outbulgings, but rather involving actual increases in thickness of the wall, which most commonly is 5 to 10 μ, at times only 3 μ, often as much as 20 μ. Wall dark, often

scarcely transparent, color ranging from somewhat golden (Mars Yellow Ridgway) to rich brown (Brussels Brown Ridg.), most commonly dark resinous amber (Sudan Brown Ridg.). Fragment of oogonial stalk frequently adherent. Oospores regularly spherical, most frequently from 23 to 28.9 μ in diameter, the mode between 25 to 26.9 μ, extremes ranging from 20 to 34 μ; wall hyaline to pale golden, 1 to 1.5 μ thick; content finely granular with aggregations of denser material and with masses of oily reserve substance, central to eccentric in position.

Germination not yet observed.

Occurring on *Sorghum plumosum* Beauv. at Glen Innes, Guyra and other points lying west of the New England range in New South Wales, in February, 1928, collected by R. J. Noble.

Specimens deposited in the Farlow Herbarium, Harvard University.

DISCUSSION

The occurrence of this new Sclerospora, as found by Dr. Noble on *Sorghum plumosum* in New South Wales, involves certain points of interest.

In the first place, this host adds another species to the long list of grasses that are attacked by the ten or more Sclerosporas in various parts of the world. Hitherto the cultivated sorghum, *Sorghum vulgare* Pers. has been the only member of this gramineous genus reported as harboring a Sclerospora. On this crop there has been reported, both from India and Africa, a species which, in its oogonial phase, resembles *Scl. graminicola,* but which, in its conidial stage, shows distinct differences that, as have been noted, distinguish it sharply from that widespread form.

Moreover, the Sclerospora itself is a species as yet undescribed, readily recognizable by the small size of its oospores (smaller than that of any other species) and their thin wall, and also by the rounded, rather than flattened, exterior of the oogonium, whose rounded surface prominences result from actual increased thickness of the dark wall, closely in contact with the oospore within, and not from out-bulgings.

In the second place, the host, *Sorghum plumosum* Beauv., the *Holcus plumosus* of R. Brown, the *Andropogon australis* subsp. *plumosus* of Sprengel, is endemic to Australia. It occurs, according to Turner (5), Bentham and Mueller (1), and others, in all the eastern colonies, and also in North Australia; very plentiful in the coastal regions along the Hasting's, McLeay, and Clarence rivers from Port Jackson to the Blue Mountains of New South Wales, and also in the New England district where, when in flower, it is reported as a noticeable feature of the landscape in the pastures.

The obligate parasitism of this endemic grass by a distinct species of Sclerospora, as yet known only on this host, may perhaps indicate that the parasite also is endemic to the region. As our knowledge of the geographic distribution and the host range of the Sclerosporas increases, it will be of interest to note whether this is verified.

In the third place, the occurrence of this Sclerospora on this grass in Australia may prove to be of economic importance. *Sorghum plumosum* is described as a valuable wild forage grass, its herbage, less coarse and heavy in the cooler parts of Australia, affording widespread fodder for grazing sheep, and its abundant seed heads furnishing a grain on which horses are said to thrive. As now known the Sclerospora is not devastatingly destructive to the wild sorghum, and it is probable that this balance is the result of long association and will not suddenly be upset. It seems probable, however, that if future conditions of stock-raising should lead to greater and greater dependence on this grass as fodder and several successive seasons with such conditions as excessive rainfall should favor unusually severe development of the fungus, it might prove of importance.

It is more probable, however, that the fungus may prove of economic importance by passing from this to other more valuable cultivated grasses. As most of the species of Sclerospora are not narrowly limited but have a considerable range through species and genera of hosts, it is to be expected that this may occur in the future, especially if changes in agricultural practice bring many gramineous crops within range of infection. The wild sorghum is perennial and the new growth in colder regions of its range starts in September to October (the beginning of "summer"), while in warmer localities it is continuous and the Sclerospora developing with it affords a constant reservoir of infection. The other species of Sorghum in Australia, *Sorghum fulvum* Beauv., occurring also in tropical Asia, and the cultivated sorghum, *Sorghum vulgare* Pers., world-wide and with many varieties, may in time contract the disease, the latter, in some of its many varieties, having possibilities as a valuable crop there, as in other parts of the world. From the behavior of other Sclerosporas it is possible this species may prove destructive to some other gramineous crop either in the Andropogoneae or the Maydeae or perhaps even some more distantly related tribe.

SUMMARY

This paper discusses a Sclerospora found by R. J. Noble in New South Wales, Australia, on *Sorghum plumosum* Beauvois, a native grass locally called "wild sorghum."

In its resting-spore stage, the only reproductive phase yet found, the oospores are of smaller size, mostly between 23 and 28.9 μ in diameter, with a thinner wall, mostly from 1 to 1.5 μ in thickness, than any known Sclerospora, while the thickness, color, and contour of the oogonial wall also mark this as a species as yet unrecorded. It is, therefore, described as *S. noblei* n. sp. and its distinguishing features of size and structure are considered in detail and compared with related forms.

As the host on which the fungus occurs is endemic to Australia the possibility is advanced that the fungus, also, may be endemic.

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CERTAIN EARLY DEVELOPMENTAL PHASES COMMON TO MANY FUNGI[1]

G. HAMILTON MARTIN[2]

For some time there have been observed organisms which were associated with diseased conditions in plants, such as wilt, scorch, yellowing, leaf-spotting, anthracnose, die-back, scab, and melanose. These organisms either have remained undetermined or have been placed in various genera that are much alike in their spore forms. The following examples are illustrative. On rose leaves there is often observed a crinkling and yellowing interspersed with areas of normal green, or, again, a red spotting of leaves, stems, petals, thorns, and hips. On the lily, iris, lilac, pear, privet, poplar, and Vinca a fire-blight effect is found. On the iris and lily a purple coloration followed by chlorosis resulting in spots distinctly grayish or whitish in dry weather and reddish brown under humid conditions has been observed. On lilac, Hydrangea, and Viburnum a blighting of shoots occurs characterized by a distinct burning or scorching of leaf and twig, after which the tissues turn brown or black and the diseased parts become brittle and persistently adhere to the plant. A study of these diseases demonstrates that the causal organisms have certain common developmental phases. Pseudosaccharomyces and Pseudofumago were used by Briosi and Farneti (5) as names of form genera. The writer regards these as mere budding and fumagoid phases of perfect stages of various fungi. It appears that the yeast-like or budding phase and the fumagoid phase play an important part in the life history of various fungi. The former phase presents less variation than the latter, as is shown later in this paper.

That the special type of growth produced is largely influenced by conditions of temperature and humidity as well as kind of media used is borne out by numerous cultural studies. Field observations also indicate that in nature these phenomena may be correlated with seasonal, weather, and soil conditions.

No doubt the budding phase which in culture presents a cloudy, slimy or watery appearance has been encountered by other workers and thought to be of bacterial origin because of its resemblance to bacterial growth. Although these fungi in their final phases are often associated with such symptoms as previously stated, there is yet no experimental evidence that they are pathogenic except when nutritional or climatic conditions affect

[1] Subsequent articles will give the details supporting the hypotheses in this paper.

[2] The writer is under obligation to Mr. J. F. Brewer for the photographic work illustrating this article.

the host so adversely as to make infection possible. Tissue decomposition is not always present in areas discolored or otherwise affected by the budding or fumagoid phases.

The Pseudosaccharomyces phase is characterized by conidia which may be borne in a whorl or produced at the apex of a terminal cell or on a protuberance which arises from any of the cells along the course of the fumagoid mycelium. The conidia are spherical to oblong-elliptical, apiculate or entire, hyaline, at times changing to brown, greenish-black, or black or they may continue to form bud cells. In the Pseudofumago phase the filaments may be hyaline, brown, greenish-black or black. They are septate and may produce papillae on which the budding spores are formed by sprout cells. At times the mycelium may become moniliform by means of segmentation; these segments may then become larger, the membrane thicker and darker. These cells are comparable to chlamydospores. Great variation may be observed in the formation of these spores. One or more cells may separate, develop thick walls. At times they divide transversely and may be easily mistaken for the spores described for the genus Coniothecium. These spores are capable of immediate germination or may function as resting spores. Various growth manifestations are exhibited by the fumagoid filaments during the transitional developmental periods which show an unusual polymorphism. The spores of Pseudofumago may either give new fumagoid chains, Pseudosaccharomyces, Cladosporium, or Hormodendron. The fumagoid phase can be obtained directly from the budding spores or from spores of Cladosporium or Hormodendron. Considerable similarity has been noticed between the development of spores in corymbs or clusters in Pseudosaccharomyces, Cladosporium, and Hormodendron. These phases are not only found in stems, leaves, flowers, seeds, or fruits but also in bulbs, rhizomes, and roots.

Certain form genera have been described which have phases that are indistinguishable from the budding or fumagoid phases. The following are some of those observed during the present investigation:

Dematium pullulans de Bary.
Fumago vagans Pers.
Kabatiella Vassil.
Polyspora Lafferty.
Protocoronospora Atk. & Edgert.
Exobasidiopsis Karak.
Pachybasidiella Bub. & Syd.
Microstroma Niessl.
Sphaceloma de Bary.
Aureobasidium Viala & Boyer.

During the course of the studies here recorded, types of Pseudosaccharomyces and Pseudofumago phases have been isolated from 50 different hosts as given in the following list:

Acer dasycarpum pyramidula
Aquilegia sp.
Begonia sp.
Buxus sempervirens
Carthamus tinctorius
Catalpa sp.
Chrysanthemum sp.
Colchicum autumnale
Convallaria majalis
Cornus sp.
Dahlia sp.
Dianthus barbatus
Funkia sp.
Fuchsia sp.
Gladiolus sp.
Gleditsia triacanthos
Hemerocallis fulva
Hicoria pecan
Hippeastrum equestre
Holcus sorghum
Holodiscus discolor
Hydrangea sp.
Iris spp.
Juglans spp.
Juniperus procumbens
Lathyrus odoratus
Lavendula sp.
Leucothoë sp.
Ligustrum ovalifolium
Lilium spp.
Maclura pomifera
Myosotis sp.
Narcissus sp.
Paeonia officinalis
Papaver orientale
Pelargonium sp.
Phaseolus lunatus macrocarpus
Philadelphus sp.
Pinus sp.
Polygonatum sp.
Populus sp.
Pyrus communis
Rhododendron spp.
Rosa spp.
Salvia sp.
Symphoricarpos sp.
Syringa vulgaris
Vicia villosa
Vinca minor
Wisteria sp.

An examination of literature shows that certain investigators have observed the budding and fumagoid phases in connection with the study of some particular organism but they appear not to have emphasized the fact that these phases are common to a large number of widely separated genera. Bennett (2) states that *Anthostomella pullulans* is the perfect stage of *Dematium pullulans*. Schwarz (15) describes and illustrates a yeast-like organism, which she designates as the A and B stages of *Graphium ulmi*. Klebahn (10) does the same for *Guignardia pullulans* on the iris and places the conidial phase in the genus Sporotrichum Link. Martin and Charles (11) found a like phase in connection with the life history of *Erostrotheca multiformis*. Fusicladium has been found by Aderhold (1) and other workers to be an imperfect form of Venturia; Brefeld (3) illustrates a budding phase for a number of Ascomycetes. His figures of the budding and fumagoid phases for *Sphaerulina intermixta* are excellent in their delineation of the polymorphism of sprout-cell formation. He calls this phase *Dematium pullulans*. It is interesting to note that Gäumann and Dodge (6) refer to this early phase of *S. intermixta* as follows: "This type of germination has not been reported elsewhere in the group and probably was based on impure cultures." This same idea has been held by a number of workers. Janczewski (7) found *Dematium pullulans*, Cladosporium and Hormodendron phases in connection with the life history of *Mycosphaerella*

tulasnei. Brefeld (4), Sadebeck (14), Pierce (13), Klebahn (9), Mix (12), and Jankowska (8) have obtained the budding or yeast-like cells in connection with cultural studies of the Exoascaceae.

An extensive cultural study by the writer of *Leptosphaeria coniothyrium* on the rose has shown that the early phases of the organism are Pseudosaccharomyces and Pseudofumago, from which pycnidial phases of Phoma, Phyllosticta, and Coniothyrium were derived. The early phases were observed to be present in the host tissue long before the canker was in evidence. The first external manifestations are wilting of leaf, shoot, and bud and red spotting on the twig, spine, leaf, petal or outer bud coat, and on the hip. The presence of the fungus is indicated by a dwarfing, a yellowing, and a distortion or wrinkling along the veins and midrib of the leaflets.

A red spotting or generalized stippling of stems, leaves, and flowers of Paeonia has been frequently reported. It is apparently what has been designated as anthracnose, a disease which Whetzel (16) conjectures to be due to a fungus producing definite though incomplete fruiting bodies. Others have supposed that it might be a physiological injury resulting from unbalanced nutrition. This type of injury was very prevalent during the spring of 1929. Many specimens were received by the writer or collected. The budding and fumagoid phases were consistently found. Similar fruiting structures were often found in abortive buds which showed symptoms like Botrytis bud blight but failed to yield Botrytis. From the stem lesions, leaf spots, and bud lesions, *Cladosporium paeoniae* developed directly on the plant and also when the budding organism was isolated in culture.

In April, 1927, species of lilies growing in Washington, D. C., were found by Vera K. Charles to be affected with a fungus that corresponded to the description of *Kabatiella microstricta* Bub. The plants presented a scorched appearance. Since then many specimens have been collected and received which, on examination, yielded an organism that corresponds morphologically and culturally with the budding and fumagoid phases dealt with in this paper. The symptoms are varied, such as grayish-white, diffused areas, scorch, wilt, rosette, abortive buds, leaf yellowing and mottling, a general blighting of leaf and bud, and a rusty appearance on bud scales and leaf. The latter condition on leaves has been called "frost injury." The organism has been isolated from all parts of the plant, including the vascular system of the bulb scales. The infection in the bulb scale is of great importance since this can be a means of spreading the disease through propagation by scales. That the organism can also be carried by the mite, *Rhizoglyphus hyacinthi*, which inhabits the bulb, has been demonstrated by taking mites from infected bulbs and placing them in tube cultures and also by examination of the mite.

During the spring of 1929, Iris in several localities was severely affected by an organism similar to that found on the lilies. It causes a general blight, a browning of the leaf tips, a scorch or wilting of the plant. A fungus of this type has been found in and isolated from the leaf, rhizome, and roots of the plant.

Pure cultures made from single spores or segments of the Pseudosaccharomycetous and Pseudofumagoid phases from various hosts have developed fruiting boidies of the following form genera. Following the name of the fungus the hosts from which such an organism was isolated are listed.

Ascochyta—*Vicia villosa.*

Cladosporium-Hormodendron—Aquilegia, Begonia, Catalpa, Chrysanthemum, Convallaria, Cornus, *Dianthus barbatus, Gleditsia triacanthos, Hicoria pecan, Holcus sorghum, Iris germanica, Juglans cinerea, J. nigra,* Lavendula, *Ligustrum ovalifolium, Lilium* spp., *Papaver orientale,* Philadelphus, Populus, *Pyrus communis,* Rhododendron, Symphoricarpos, *Syringa vulgaris, Vicia villosa,* Vinca, Wisteria.

Colletotrichum—Hydrangea, Paeonia, Symphoricarpos.

Coniothyrium—*Gleditsia triacanthos,* Paeonia, Rosa.

Fusicladium—*Pyrus communis.*

Gloeosporium—Hydrangea, Paeonia, Symphoricarpos.

Kabatiella microstricta—Convallaria, Iris, Lilium.

Kabatiella nigricans—*Vicia villosa.*

Kabatiella polyspora—*Acer dasycarpum pyramidula.*

Marssonina—Aquilegia.

Microstroma juglandis—*Hicoria pecan, Juglans cinerea, J. nigra.*

Phoma—Rosa, *Syringa vulgaris, Vinca minor.*

Phyllosticta—Aquilegia, *Dianthus barbatus,* Hydrangea, *Hicoria pecan, Holcus sorghum, Hippeastrum equestre, Iris germanica, Ligustrum ovalifolium,* Paeonia, Polygonatum, Rosa, *Vicia villosa,* Vinca, Wisteria.

Sclerotium—Lilium, Convallaria, Iris.

Glomerella cingulata (Ston.) Spauld. & Schrenk—Hydrangea, Paeonia, Symphoricarpos.

Pleosphaerulina sp.—*Hippeastrum equestre, Holcus sorghum.*

SUMMARY

Budding or yeast-like cells have been observed associated with diseased conditions such as wilt, scorch, yellowing, leaf spotting, anthracnose, dieback, scab, melanose, rosette, dwarfing and shoot blighting on fifty different hosts. A study of these diseases demonstrates that the causal organisms have common developmental phases which play an important part in the life history of various fungi. The terms Pseudosaccharomyces and Pseudofumago are used for the early phases. A description is given for each.

Mention is made of a number of instances in literature where the budding and fumagoid phases have been observed in connection with the study of some particular organism.

Species of Ascochyta, Cladosporium-Hormodendron, Colletotrichum, Coniothyrium, Fusicladium Gloeosporium, Kabatiella, Marssonina, Microstroma, Phoma, Phyllosticta, Sclerotium, *Glomerella cingulata,* and Pleosphaerulina have been developed from single spores or segments of Pseudosaccharomyces and Pseudofumago in culture.

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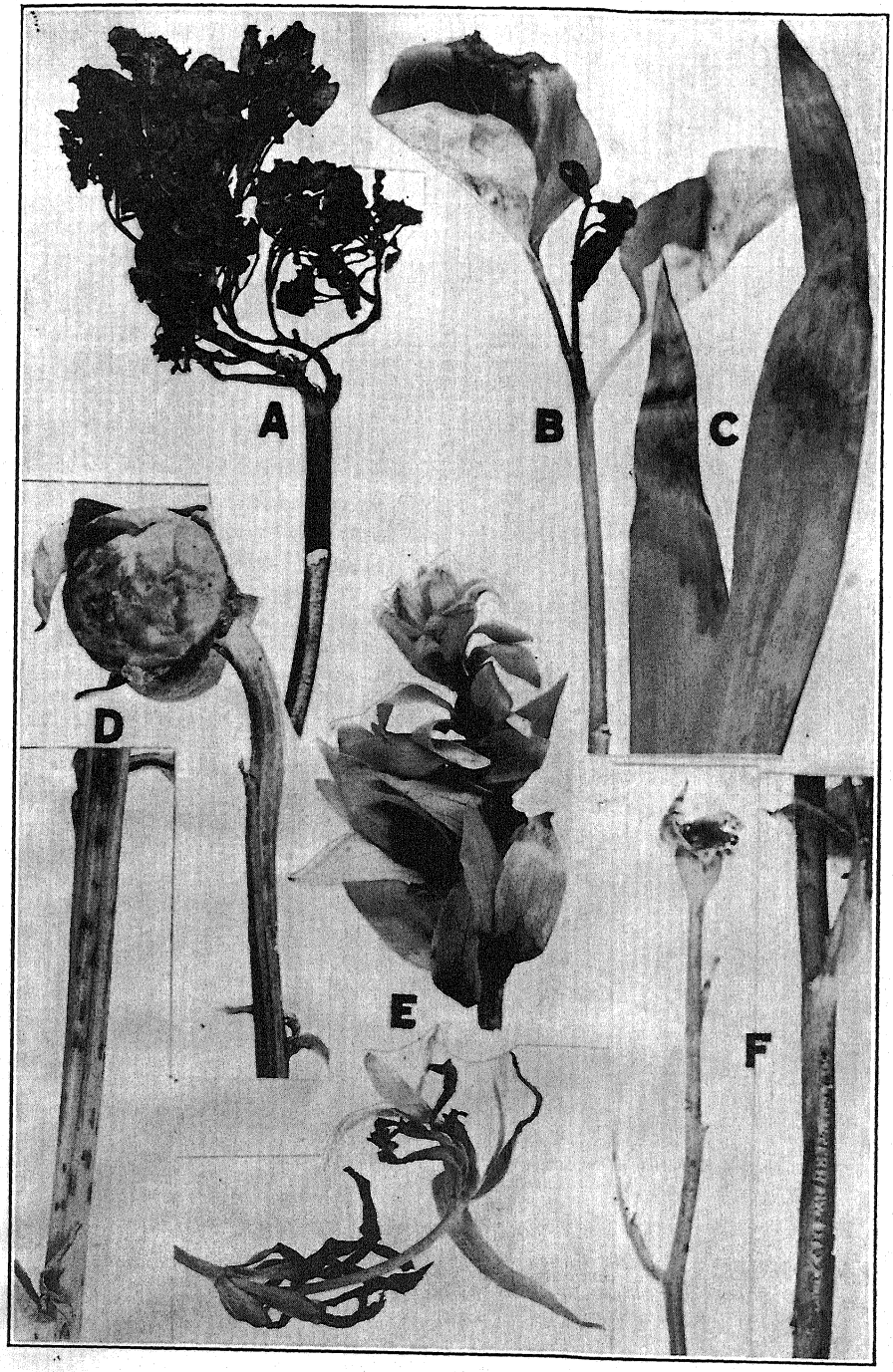
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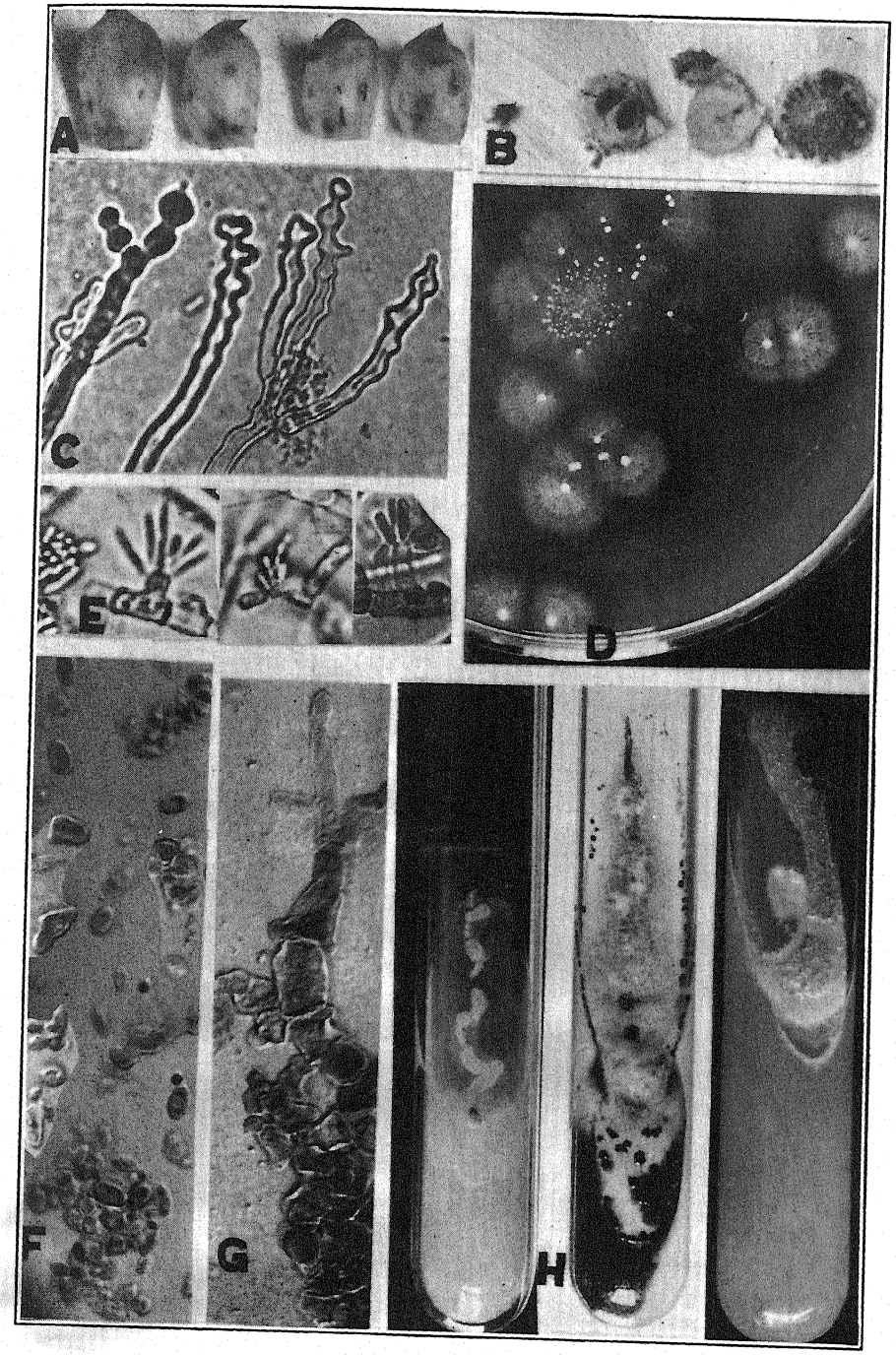
PLATE XXXVII

A. Blossom blight of Hydrangea.
B. Shoot blight of lilac.
C. Scorch of Iris.
D. Anthracnose and bud blight of peony.
E. Scorch of lily.
F. Spotting and young lesions caused by *Leptothyrium coniothyrium* on the rose.

PLATE XXXVIII

A. Natural infected bulb scales of lily by *Kabatiella microstricta.*
B. Cross-section of lily bulb showing vascular infection.
C. Photomicrograph of young, moniliform filaments of Pseudofumago from *Lilium* sp.
D. Petri-dish culture of colonies of budding spores from *Lilium* sp.
E. Whorl or cluster formation of budding spores on fumagoid filament from *Lathyrus odoratus.*
F. Pseudosaccharomycetous spores.
G. Pseudofumago mycelium from *Lathyrus odoratus.*
H. Cultures of budding phase resembling bacterial growth from *Lilium* sp.


A
B
C
D
E
F


A
B
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H

A TWIG CANKER OF APPLE CAUSED BY *NECTRIA CINNABARINA*

H. E. Thomas and A. B. Burrell

Early in the summer of 1927 the attention of one of the writers was drawn to an unusual blight of Rome apple twigs which, at a distance, somewhat resembled fire blight. It was found, however, that the death of the terminals was caused by small cankers originating in the cluster bases which had borne fruit the preceding season. Specimens taken in August, 1928, bore numerous sporodochia and conidia of a fungus indistinguishable from *Nectria cinnabarina* (Tode) Fr. (*Creonectria purpurea* (L.) Seaver). Thus far the canker has been seen by the writers in only a single orchard near Cardiff, in Central New York.

The cankers were fairly numerous and wide-spread on the Rome variety and were found in appreciable numbers on Northern Spy. None was found on McIntosh interplanted with the Romes or on Delicious or other varieties in neighboring blocks. *Nectria cinnabarina* was abundant in a planting of currants a few rods distant from the Rome trees. It is interesting that Durand records the occurrence of the same fungus on the pear in the same county in 1897.[1] *Nectria cinnabarina* has been found associated with the death of twigs and branches of many sorts of trees, including the apple in Oregon,[2] in New Zealand,[3] and apparently elsewhere, but experimental evidence of its causative relation in the case of the apple has not been found. Indeed, most of the infection experiments with this fungus on other plants have been attended either by failure or indifferent success. R. J. Haskell reports (letter of September 1, 1928) two collections of the conidial stage of this fungus on apple in the mycological collections of the Bureau of Plant Industry, one from Maine on a fruit spur and another from Washington, taken from a larger branch.

The disease is as yet of no great economic importance in the orchard under observation, although a maximum of several hundred dead fruit spurs may be found on a single tree.

The superficial resemblance of the symptoms of this disease to those of fire blight on twigs has been mentioned. The foliage and bark, however, do not assume the dark aspect associated with fire blight but rather a light

[1] Durand, E. J. A disease of currant canes. N. Y. (Cornell) Agr. Exp. Sta. Bul. 125. 1897.

[2] Zeller, S. M. Cankers of apple and pear in Oregon and their control. Oreg. Agr. Exp. Sta. Circ. 73. 1926.

[3] Cunningham, G. H. Coral-spot, Nectria cinnabarina (Tode) Fries. New Zealand Jour. Agr. 25: 354–359. 1922.

brown color. The bark and wood are killed from the tip of the cluster base downward to a distance of one to four inches. Branches arising from the cluster base are girdled and killed. Early in the season there are no conspicuous signs of the fungus but, later, the bright pink stromatic masses or sporodochia are borne in profusion on the dead bark, particularly that of the cluster base (Fig. 1).

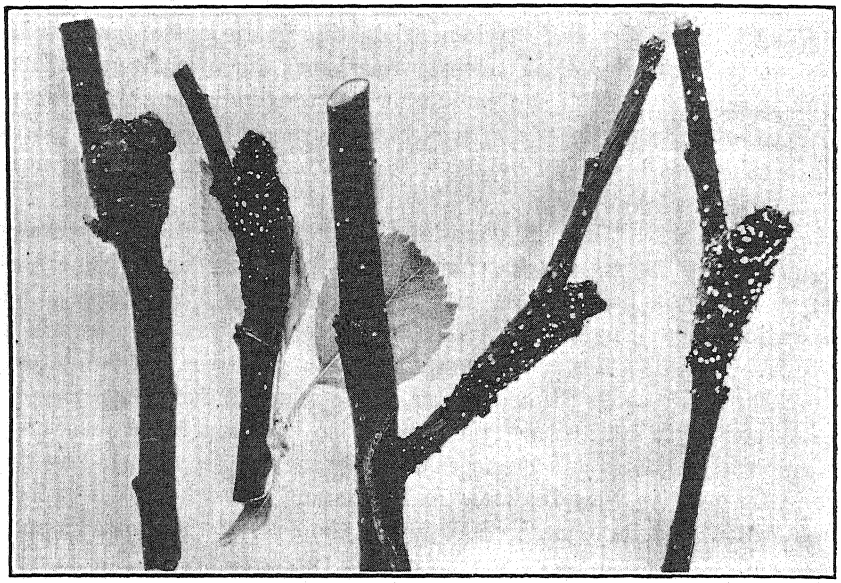


FIG. 1. *Nectria cinnabarina* on Rome apple fruit spurs. Collected in August.

In addition to the sporodochia already referred to, perithecia with viable ascospores were found on material collected on March 1, 1929. These agree in all essential details with the written descriptions of *N. cinnabarina.*

It was soon noted, however, that cultures from the cankers on Rome twigs differed in certain respects from those obtained from currant bushes in a garden at Ithaca. This led to a more extended comparison of some of the strains[4] from apple and currant obtained from ascospores, and conidia. Representative strains were grown on potato, potato-dextrose, oat, beef-peptone, and Czapek's[5] agars. Most of these cultures were made from two strains of the fungus, one from apple and one from currant. Good growth was obtained on all these media and on steamed apple twigs. Differences were observed between strains in growth rate, type of colony, and the num-

[4] The term strain is used to designate the line originating from a separate isolation and does not imply that differences exist between the lines so designated.

[5] Reed, H. S. A manual of bacteriology. Boston, 1914.

ber of sporodochia produced. In some instances the differences were striking but sufficient work has not been done to determine how nearly constant these differences may be. However, 30 strains from ascospore-dilution cultures all from the same source were indistinguishable on potato-dextrose agar with the exception that one strain produced sporodochia somewhat more readily than the others of this group. Twelve of these strains were matched in Petri-dish cultures, two strains to each culture. A slight antagonism was shown between colonies which was similar throughout these and other cultures. No other effect of matching was noted at the end of eight weeks.

The temperature relations of a strain from apple were studied on potato and Czapek's agars at 3, 9, 15, 21, 27–29, and 33 degrees Centigrade. Growth was slight at 3° and best at 21°. Moderate growth occurred at 27–29° and no growth at 33° C.

INFECTION EXPERIMENTS

Inoculations were made in the greenhouse on young apple trees of the varieties McIntosh and Wealthy and on grafted fruit spurs of the Rome, Northern Spy, and Winter Banana varieties. The inoculum was taken from agar cultures (originally from conidia) in the first trial and from cultures (ascospore from apple) on steamed wheat kernels in the second and third trials. The inoculations were made on wounds produced by heading back the terminals or by slitting the bark on the stem. In the case of the fruit spurs, the cluster base was split at the tip for a distance of one-fourth to one-half inch, making a rather severe wound. Following inoculation, the trees were maintained in a moist atmosphere for 5 to 7 days.

In the first experiment, 6 wounds on McIntosh and Wealthy trees of low vigor were inoculated with each of two strains of the fungus, one from apple and one from currant. No infection resulted.

Again, 4 Wealthy and 8 McIntosh trees growing in a greenhouse bed were inoculated when the first leaves were one-half inch to one inch long. Two inoculations were made on each plant. Growth in these plants, after 52 days, varied from a few clusters of leaves to the production of shoots 12 to 16 inches long. Of the 4 inoculated Wealthy trees, one tree died apparently due in part to causes other than the inoculation. Two of 8 uninoculated Wealthy trees died before the trees became rooted in the soil. In the three remaining inoculated Wealthies the fungus had advanced into the living tissues from two wounds but at most only one-half inch from the wound. The 8 inoculated McIntosh trees showed much more marked evidence of pathologic reaction. Five of them died outright and bore numerous sporodochia of the fungus. One plant produced a shoot 7 inches long

before it succumbed. The sixth inoculated plant bore a canker one-and-one-half inches long, while the two remaining plants showed only slight killing of the bark around the wounds. One of 8 uninoculated McIntosh trees died.

Inoculations on the fruit spurs were made within a few days of the time of grafting and are somewhat inconclusive in view of the rather severe wounding and the fact that 50 per cent of the uninoculated grafts failed to develop. However, it is of interest that on the Rome spurs the progress of the fungus down the stems was much more rapid than on Northern Spy or Winter Banana. Fifteen of twenty-four inoculated Rome spurs were entirely dead in 33 days and two more were nearly so. Only one of three inoculated Winter Banana spurs was dead and none of four Spy spurs was entirely so. There was evidence of killing beyond that attributed to the wounds on all except four of the 31 inoculated spurs.

Natural infection in the field apparently takes place in late autumn or early spring. The infection court is quite obviously at or near the point of detachment of the fruit from the cluster base. Ascospores were mature in 1929 in considerable numbers by March 1. On April 7, many cankers were found dead from the tip of the cluster base to a distance of a few millimeters to two or more inches. Observations indicate that there are few if any new infections developed during the summer, although conidia are produced in great profusion from midsummer on. The cankers of one season usually do not persist into the next.

The disease seems to be most abundant on trees of moderate vigor and less common on trees of very low or very high vigor.

SUMMARY

1. *Nectria cinnabarina* is consistently associated with a canker on apple twigs in one orchard in central New York.

2. Natural infection takes place at or near the point of detachment of the fruit from the cluster base.

3. From inoculated wounds the fungus is able to invade the tissues of several varieties of apples in degrees varying with the variety and with the growth condition of the plant.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

STEM-RUST-RESISTANT SEGREGATES FROM WHEAT CROSSES BETWEEN TWO SUSCEPTIBLE PARENTS[1]

GEORGE STEWART[2]

In addition to valuable economic forms, the breeding program to secure wheat strains resistant to stem rust is constantly yielding interesting scientific data in regard both to the rust organism and to the host plant. Some resistant segregates have been obtained at the Utah Agricultural Experiment Station from crosses between parents, one of which is fully susceptible and the other only mildly resistant. A recombination which gave semiresistant segregates from two susceptible parents has been reported by Hayes, Aamodt, and Stevenson.[3] From a cross between Federation, which is fully susceptible (100S), and Sevier No. 59, a pure line that is weakly semiresistant (75SR), the writer obtained and reported a fully-resistant segregate (R type of resistance).[4] Out of 18 segregates tested, 13 were fully susceptible (70–90S), 4 were semiresistant (50–65SR), and one was fully resistant (25R). Several other segregates had previously been obtained[5] when Sevier was one of the parents, but the reaction of the parental pure line of Sevier was not known.

A second fully-resistant segregate (R) was obtained in 1928, in which one parent, Federation, was completely susceptible (60S) and the other, Sevier No. 59, was of the semiresistant type. The breeding was done in 1924, 1925, and 1926 at Logan, Utah, where homozygous F_4 lines were secured in 1927. The rust tests were made in the rust nursery at St. Paul, Minnesota, in 1928.[6] In all, 26 strains were tested. Of these, 12 produced rust reactions of the purely susceptible type; 11 gave reactions of the semiresistant type or segregated for the susceptible and semiresistant types; 2 strains segregated for the semiresistant and resistant types of reaction; and

[1] Contribution from Department of Agronomy, Utah Agricultural Experiment Station.

[2] Agronomist in charge plant breeding. Publication authorized by Director of the Utah Agricultural Experiment Station, May 21, 1929.

[3] Hayes, H. K., O. S. Aamodt, and F. J. Stevenson. Correlation between yielding ability, reaction to certain diseases, and other characters in spring and winter wheats in rod-row trials. Jour. Amer. Soc. Agron. 19: 896–910. 1927.

[4] Stewart, George. Origin of a segregate resistant to blackstem rust in a cross between two susceptible parents. Amer. Nat. 62: 188–192. 1928.

[5] Stewart, George. Correlated inheritance in wheat. Jour. Agr. Res. 33: 1163–1192. 1926.

[6] The writer gratefully acknowledges his indebtedness for the rust readings to the following: Dr. H. K. Hayes, Head, Division of Agronomy, Minnesota Experiment Station; Dr. E. C. Stakman, Head, Section of Plant Pathology, Minnesota Experiment Station; and K. S. Quisenberry, Associate Agronomist, U. S. Department of Agriculture, quartered at Minnesota Station cooperating on rust studies.

one strain was fully resistant (15R). The Federation parent was again completely susceptible (60S) and the Sevier No. 59 parent was semiresistant (50SR). The data are presented in table 1.

TABLE 1.—*Percentage of stem-rust infection on parental strains of wheat and on segregates which gave infection reactions ranging from fully susceptible (S) to fully resistant (R). Also the percentage of leaf-rust infection of segregates grouped according to their reaction to stem rust*

Parents or segregates	Susceptible reaction (S)	Semi-resistant reaction (SR)	Segregating for S and SR	Segregating for SR and R	Resistant reactions (R)
Federation					
Stem rust	60				
Leaf rust	60s				
Sevier No. 59					
Stem rust		50			
Leaf rust		75s			
Federation×Sevier No. 59					
Stem rust					
No. of segregates	12	7	4	2	1
Range in infection	30–70	30–60	50–60	30–50	
Mean	58	49	55	40	15
Leaf rust	60–90s	70–85s	75–85s	75–85s	90

Table 1 establishes a segregation for resistance types of reaction to stem rust. By the F_5 generation 12 of 26 strains were homozygous for full susceptibility (S); 7 were homozygous for semiresistance (SR); and 1 for complete resistance (R). Six of the 26 strains were still segregating for rust reaction. Four strains segregated in 1928 (F_5) for complete susceptibility (S) and semiresistance (SR); two strains segregated for semiresistance (SR) and resistance (R). One of these gave a reading of 30 R-SR.

Observations also were made on leaf rust, but all the segregates studied were susceptible (S). The segregate resistant to stem rust was highly susceptible to leaf rust, the reading being 90s. The two strains segregating for resistance and semiresistance to stem rust gave readings for leaf rust of 85s and 75s when the readings for stem rust were 30R-SR and 50R-SR, respectively. With every leaf-rust reading higher than 60s, the data available do not show whether there is linkage between the reaction for stem rust and leaf rust as Hayes, Aamodt, and Stevenson[3] thought.

However, it is clear that out of 26 strains, three were more resistant than either parent. One of these was homozygous for a high type of resistance (15R).

UTAH AGRICULTURAL EXPERIMENT STATION,
LOGAN, UTAH.

THE EFFECT OF LAND PLASTER APPLIED AS A DUST ON SEED CORN

E. N. Bressman

In recent years there has developed no little interest in the use of dust fungicides on seed corn. Most of the commercial dusts are organic mercury compounds and are used primarily to control disease and stimulate the growth of corn. Early in the spring of 1926 the writer's attention was called to the use of land plaster (powdered gypsum), as a dust on seed corn, by a grower in Western Oregon. Land plaster has long been used and known as a crop stimulant, but its use as a dust on seed corn for the control of mold fungi which seem detrimental to germination and to seedling growth, is a rather new idea. It is commonly added to the soil in Western Oregon as a fertilizer for legumes.

Germinator trials were conducted. These showed that seed treated with land plaster germinate more quickly and develop a larger and more vigorous root system than untreated seed. Figure 1 shows treated and untreated

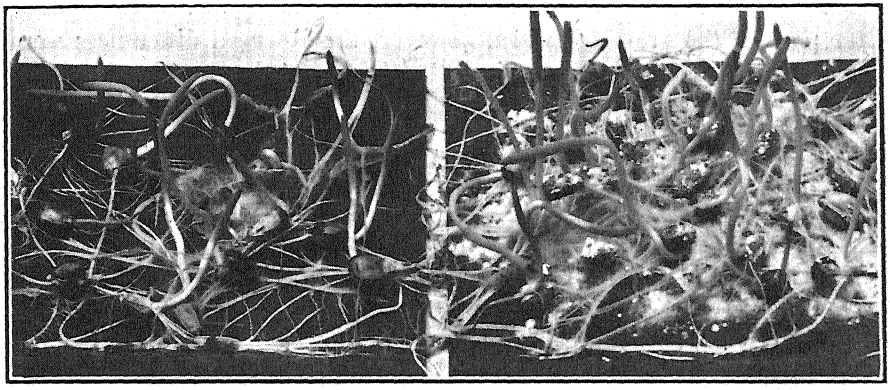


Fig. 1. Seed corn after 5 days in a standard germinator. Untreated (left) and treated (right).

Minnesota No. 13 seed corn five days after it was placed in the germinator.

In the field trials 8 pounds of commercial land plaster per acre were used. The corn was planted in hills at the rate of 5 kernels per hill and land plaster was dusted on the seed by hand before the hills were covered. After the corn had attained a height of about 5 inches, it was thinned to 3 plants per hill. The hills were spaced 3 feet, 4 inches apart each way. The plots were 1/35-acre in size. Only single plots were grown in 1926 and 1928. Duplicate plots were grown in 1927. For silage yields the entire plot was

harvested and weighed. The ears were then husked from this silage corn and weighed. In each of the three annual trials the larger growth and darker green color of the treated plots could easily be seen throughout most of the growing season. Figure 2 shows the plots in 1927. During the lat-



FIG. 2. The three rows of corn at the left are from seed treated with land plaster. The three rows at the right received no treatment. Corvallis, Oregon, 1927.

ter part of the growing season, however, the observed differences were not great. The following table gives the results of three annual trials in the field.

TABLE 1.—*The effect of land plaster on yields of Minnesota No. 13 corn at Corvallis, Oregon. Yields given in bushels of ear corn and tons of silage per acre*

	1926		1927		1928		Average	
Treatment	Bu.	Tons	Bu.	Tons	Bu.	Tons	Bu.	Tons
Land plaster on seed	61.0	6.5	58.5	6.2	48.0	5.5	55.8	6.1
No treatment	54.3	6.7	50.0	5.9	34.5	5.2	46.3	5.9

Several other dust treatments were used at various times in these experiments. Copper carbonate, Semesan Jr., and Uspulun gave slightly increased yields. Bayer's dust, Trockenbeize Höchst, Bayer's Compound, and Bayer's Spec. No. 169 gave decreased yields. All of the above dusts, used in excess, injured the germination of the seed. Land-plastered seed gave higher yields than that treated with any other dust.

Various amounts of land plaster, from one to nearly fifty pounds per acre, were used in additional trials. Stimulation of growth was obtained in all trials and no injury resulted. In addition to stimulating the growth

of corn, the land plaster eliminates some of the molds which are usually found in the germinator. Other molds are not controlled.

Land plaster does not readily adhere to the seed. A fertilizer attachment on the corn planter, which places the land plaster in contact with the seed in the soil, is the best means of application.

AGRICULTURAL EXPERIMENT STATION,
OREGON STATE AGRICULTURAL COLLEGE,
CORVALLIS, OREGON.

REPORT OF THE THIRTEENTH ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

OFFICERS

President ..... J. W. HOTSON, University of Washington, Seattle.
Vice-President ..... EUBANKS CARSNER, University of California Citrus Experiment Station, Riverside.
Secretary-Treasurer ..... B. A. RUDOLPH, University of California Deciduous Fruit Station, San Jose.
Councilor ..... J. T. BARRETT, University of California, Berkeley.

The meetings of the Pacific Division of the American Phytopathological Society were held in conjunction with those of the Pacific Division of the American Association for the Advancement of Science and Affiliated Societies at the University of California at Berkeley, June 19–22, 1929.

A short business meeting preceded the presentation of papers. In conformance with the requirements of the constitution, an amendment to the latter which provides for the automatic elimination of members whose dues are in arrears for two years, was passed by a unanimous vote of all members present.

The very excellent paper of Dr. Ruth F. Allen, a brief abstract of which accompanies the present report, was considered the most outstanding piece of research work presented before the society this year, from the standpoint of completeness as well as for its far-reaching importance. Because the paper conforms very closely to the requirements laid down by the Committee on Awards of the Pacific Division of the American Association for the Advancement of Science, the society has submitted Dr. Allen's paper to the committee in competition for the annual prize.

During the meetings moving picture films of members of the American Phytopathological Society were shown. These same films, which were shown at the annual meetings of the parent society in New York last winter, proved a source of much amusement and pleasure at the meetings of the Pacific Division.

There was an average attendance of forty at the Berkeley meetings, and thirty papers were presented, abstracts of which follow.

B. A. RUDOLPH, *Secretary-Treasurer.*

ABSTRACTS

Phoma terrestria, n. sp., as the cause of pink root of onions.—H. N. HANSEN.

The pink root disease of onions has for many years been attributed to species of Fusarium, *i.e.*, *Fusarium malli* Taub., *Fusarium cromyopthoron* Sideris and other species. The writer was unable to produce this disease with these and other species of Fusarium

under controlled conditions. He did, however, readily produce the disease with a species of Phoma which he isolated from infected onion roots obtained from various localities in California and Texas. The name **Phoma terrestria**, n. sp., is suggested for this fungus.

The relative resistance of varieties and species of Citrus to bark diseases.—L. J. KLOTZ and H. S. FAWCETT.

Results of inoculation experiments with *Phytophthora* (Pythiacystis) *citrophthora* into 78 varieties and species of Citrus have confirmed previous observations and extended the information regarding many not previously observed. A method to secure uniform inoculations was worked out. The area of bark killed used as a measure of susceptibility.

The varieties and strains of lemon were all very susceptible. Sour oranges were very resistant. Commercial varieties of grape fruit and sweet oranges averaged as groups showed susceptibility about equal to each other but much less than lemon. As a group the pummelo varieties exhibited great variability, from a resistance only slightly less than that of the standard sour orange to a susceptibility approaching lemon. Variability in the sweet orange group was less than in the pummelo group. The mandarin was much below the pummelo or sweet orange, being similar in resistance to rough lemon and to the limes. The two varieties of citron inoculated showed pronounced resistance. The citranges—Sanford, Savage, and Cunningham—were resistant.

Experiments in the inhibition of the enzymes by the barks of the several varieties indicate the possibility of securing a biochemical test for resistance along the line indicated by Klotz in Science n.s. 66: 631–632. 1927.

On some limiting factors in the use of saturated petroleum oils as insecticides on living plants.—HUGH KNIGHT, JOSEPH C. CHAMBERLIN, AND CHAS. D. SAMUELS.

The scalecidally effective, but chemically inactive, saturated petroleum oils of viscosity 75–110 seconds Saybolt, recently employed successfully in competition with HCN fumigation for control of the red and black scales of Citrus in California, have been studied from the standpoint of dynamic phytotoxicopathy.

It was experimentally ascertained that these oils initiated metabolic disturbances which were reflected in decreased rates of transpiration, photosynthesis, downward carbohydrate translocation and in an increased rate of respiration. These effects, as well as rate of recovery, are positively correlated with and proportional to the viscosity of the oil. Phytometabolic normalization was found to be dependent upon oil elimination. Initial disappearance from the epidermis of the leaf results from intercellular absorption, the rate being a function of oil viscosity and epidermal and vascular morphology. *Intracellular* absorption next occurs followed by translocation *via* the phloem and medullary rays to apparently permanent storage in cells of the pith and old xylem. Oil translocation is a lengthy process and for more viscous types probably is never complete, thus serving to maintain a chronic condition of metabolic instability or abnormality.

The employment of oils of 60 seconds viscosity or less for practical scale control is strongly indicated.

Life history of Sclerotinia sclerotiorum (Lib.) Mass., in connection with the green rot of apricots.—RALPH E. SMITH.

This is a rotting of the young fruit of the apricot caused by the fungus mentioned, which, in seasons of favorable weather conditions, causes infection through the old calyces clinging to the surface of the fruit. No spore form of the fungus is known to occur on the tree. Its distribution depends on ascospores produced in apothecia from

sclerotia in the soil. The common idea has been that wet weather favoring fruit infection likewise induces ascospore production in orchard soil beneath the trees.

The dry, cold winter and early spring of 1929 were very unfavorable to this disease. Not a single case of green rot was observed and neither apothecia nor mycelium was found after observations were commenced on March first. Blossoms and young fruits of apricots and other fruit trees from many different places, various wild flowers, and other vegetation were collected at weekly intervals and placed in moist chambers. Apricot blossoms were just beginning to open when this was started. By this method it was found that *Sclerotinia sclerotiorum* was universally present on all sorts of vegetation in central, interior California between March 1 and May 1. No imperfect stage of the fungus was found except the occasional production of germinable gonidia. It would appear that ascospores or some other reproductive form of the fungus had been produced and widely distributed at some time before the observations were first made.

Influence of spindle-tuber disease on the physiology of the potato tuber.—WALTER JONES and T. E. RAWLINS.

Most living tissues of healthy tubers were found more highly resistant to the passage of an electric current than were similar tissues of diseased tubers. The difference in resistance is apparently insufficient to warrant the use of the resistance method by the grower for separating diseased from healthy tubers.

Juice from healthy tubers was found to have a higher resistance than from diseased tubers, indicating a higher concentration of electrolytes in the latter.

No distinct difference was found in the dry weight of ash content of diseased and healthy tubers.

Mass action in relation to infection with special reference to curly top of sugar beets.—EUBANKS CARSNER and C. F. LACKEY.

The fact that the quantity of the inoculum or dosage of the causal agent is generally one of the determining factors in infection is brought out by a review of the limited amount of literature dealing directly with this subject and by reinterpretation of pertinent data taken from a wide range of sources. The principle involved in this relationship is essentially the same as that manifested in the phenomena called allelocatalysis, spore-load effect, and synergistic or communal activity. The term mass action is preferred because, according to the hypothesis here advanced, the result of an inoculation, that is to say, infection or noninfection and usually the length of incubation period, depends, so far as it is determined by mass action, on the proportions of the chemical substances actively entering into the reaction.

The relation of mass action to infection is shown to hold in curly top as follows: (1) By varying the amount of inoculated virus by (a) inoculations with contrasting numbers of leaf hoppers and (b) unequal periods of exposure; (2) by use of plants differing in susceptibility; (3) by studies on the incubation of the virus in the insect; and (4) by comparing the minimal infective doses of the virus in its virulent and attenuated conditions.

The demonstration of mass action as a factor in curly-top infection makes clearer the significance of the observed relationship between time of planting and extent of damage in sugar-beet fields. It supports the claim that the amount of damage is positively correlated with the number of invading leaf hoppers. The feasibility of controlling the amount of virus renders the mass-action factor useful as a rough measure of curly-top resistance. It is argued that the demonstration of the relation of mass action to infection in curly top supports the theory that the virus is some sort of living organism.

Discovery of the perfect stage of a Phomopsis on lemon bark.—H. S. FAWCETT and C. L. SHEAR.

A Diaporthe was discovered on the bark of lemon trees affected with decorticosis at North Whittier Heights in March, 1928. (Ann. Rept. Calif. Agr. Exp. Sta. 1927–1928, p. 42.) Pure-culture tests and inoculations have shown it to be the perfect stage of *Phomopsis californica* Faw., which had previously been described as the causal agent of decorticosis of lemon trunks and stem-end rot of lemon fruits (Phytopath. 12: 419–424, 1922). The perithecia are embedded in the surface layers of the dead flakes or scales of bark.

Occurrence and insect transmission of Nematospora in California.—HOWARD S. FAWCETT.

The substance of this paper has appeared in Phytopathology 19: 479–482, in which a note by Gardner (Phytopath. 14: 31) on Nematospora in Lima beans was overlooked. *Nematospora Coryli* was discovered in pomegranate and Citrus fruits and in cotton bolls in Imperial County, California, in the fall of 1928. It was found widely distributed within the Imperial Valley on cotton bolls. Experiments have confirmed the first observations indicating that the fungus was being carried by the Western leaf-footed plant bug, *Leptoglossus zonatus,* from pomegranate to Citrus fruits. Pomegranate appeared to be a new host for Nematospora and *Leptoglossus zonatus,* a new carrier. Pure cultures on glucose-potato agar are yeast-like, resembling in growth that described by Ashby and Nowell (Ann. Bot. 40: 69–83, 1926). The fungus produces in pomegranates depressed spots in the fleshy covering of the seeds followed by browning and collapse of the pulp, in Citrus brown or reddish brown stains and drying out of juice sacs with premature coloring and dropping, and in cotton bolls yellow or reddish brown discoloration of the lint and seeds.

Descriptions and illustrations of injury to cotton bolls in previous entomological literature indicate strongly that the fungus has been present for a long time in Imperial Valley and possibly in Arizona and Northern Mexico.

A preliminary report of a digest of the world's literature on hadromycosis due to Verticillium spp.—B. A. RUDOLPH.

Verticillium hadromycosis is a disease to which a great group of the most widely unrelated dicotyledonous plants are susceptible. The literature on the subject is in a very chaotic condition. New hosts are being reported constantly. The host indices and bibliographies which have appeared are very incomplete and unsatisfactory. A bibliography and a digest of the literature up to and including 1928 have been prepared. A host index which is based upon authentic records includes one hundred and forty distinct species distributed in thirty-five families and eighteen orders. The history of the disease in each species has been given briefly. It is hoped that such a paper, soon to be ready for publication, will be helpful to professional plant pathologists.

Leaf and scape spot of the Amarillidaceae in California.—C. O. SMITH.

The cause of this trouble is *Phyllosticta narcissi* Anderh. It causes red spots on Amaryllis (Hippeastrum) and dead areas followed by yellowing on Narcissus. This organism has been isolated from each of these hosts and successful inoculations and cross inoculations have been made. In this study a different form resembling *Stagonosporium curtisii* (Berk.) Sacc. was found. A study of this form and *Phyllosticta narcissi* indicates that the two are probably identical. When puncture inoculations on Narcissus and Amaryllis are made with *Phyllosticta narcissi* the pycnidia often contain spores that are variable in size, shape, and septation. A single pycnidium may have large 1–3 septate spores and small continuous ones, as shown by the following:

1-cell spores measure	4.5–10 x 3 –4.5 μ
2-cell spores measure	8–13 x 5 – 6 μ
3-cell spores measure	12–18 x 4.5– 6 μ

In dilution plates from these septate spores seven subcultures have been studied and from them successful artificial inoculations have been made. All of these subcultures on sterilized Narcissus stems show the Phyllosticta type of fruiting.

It is believed that the size and septation of these spores may be a stage of maturity just preceding spore germination induced by certain favorable conditions. The larger Stagonosporium-like spores germinate within 3 to 4 hours, while the small continuous spores require 7 to 9 hours.

The Stagonosporium form appears on tissue inoculated with *Phyllosticta narcissi* from Amaryllis and Narcissus. Cultures made from the Stagonosporium fungus will again develop the Phyllosticta type. They are evidently different forms of the same fungus.

Diamond canker of the prune.—RALPH E. SMITH.

This disease of the French prune causes a pronounced roughening and swelling of the bark on the trunk and main limbs. The disease develops very slowly and gradually; in its early stages the quantity and size of the fruit are actually increased but eventually the tree becomes stunted and dies. The canker originates in the phellogen and consists mainly of a thick, leathery, heavily suberized mass of cork cells, much like the normal outer bark of many trees.

The disease has been observed only on the French prune and the variety Standard. There is considerable evidence that the Imperial, Sugar, and Robe de Sergeant prunes, other plums, and the almond, peach, and apricot are immune from diamond canker.

This condition has been thought to represent either a distinct strain or a systemic disease of the French prune, intentionally propagated and distributed by a certain nursery because of the increased size of the fruit. Investigation reveals little to support this idea but rather points to the conclusion that the disease is caused by a slow-acting fungous or bacterial infection initiated where callus formation is taking place, *i.e.*, wounds, bark cracks, or pruning cuts. Cultures and inoculations do not readily reveal the cause of the disease which in many ways resembles the so-called scaly bark (psorosis) of citrus trees. A fungus resembling *Dematium pullulans* d. By. is very regularly isolated from the cankers, together with a species of Actinomyces and a small Bacillus.

Resistance to Septoria tritici in wheat.—W. W. MACKIE.

Septoria tritici Desm. causes what is commonly called speckled blotch because of the prominent black pycnidia usually characteristic of the mature lesions. It is distinguished from glume blotch (*Septoria nodorum* Berk.) because its attacks are confined solely to the leaves. The disease is common in northern California and, in wet years throughout the State. It is especially prevalent in late wet spring seasons. It survives in the old diseased straw in the form of pycniospores in the old pycnidia. These spores are distributed by the wind and scattered upon young wheat seedlings by the first rains. A generation from spore (pycniospore or conidiospores) to spore requires from 12 to 15 days. Young seedlings are severely attacked and many killed. Under favorable conditions the disease continues until the dough stage, weakening the plants and causing them to lodge. The grain is shriveled. Tests with many hundreds of varieties yielded no immune varieties in the common bread wheats (*Triticum vulgare*) but many in the other species of wheat. By repeatedly crossing the most resistant selections of hybrid stocks, Septoria-free plants were produced. These in turn were crossed with susceptible

plants in F_1, and three susceptible to one resistant in F_2. In F_3 278 families (plant to row test) gave a very close approximation to a 3:1 ratio of susceptibility to resistance.

Tuber transmission of psyllid yellows in California.—MICHAEL SHAPOVALOV.

Although the tomato psylla (*Paratrioza cockerelli* Sulc.) has long been known to exist in California, psyllid yellows on potatoes was not discovered until the fall of 1928. At that time some badly diseased fields were found and several young tubers collected and planted out-of-doors and in pots at Riverside. One mixed lot, taken from different hills in a severely-attacked field, produced plants in part affected by yellows; some also showed mosaic symptoms, and part were free from this disease. Another lot, the progenies of several hills kept separately, showed different stages of the disease in plants grown from tubers of different hills. Furthermore, it was observed in the spring of 1928 that fields planted with potatoes grown in the psyllid-infested area showed a considerable amount of the disease in the absence of psyllids. The symptoms, however, were very much more pronounced in dry and hot interior sections than on the Coast or when artificially shaded. These data indicate: (1) At least in some localities psyllid yellows may be transmitted with the tubers; (2) the progeny may show various stages of the disease; and (3) psyllid yellows and mosaic have distinct and specific effects when present in the same plant.

Pythiaceous root parasites of various agricultural plants.—C. P. SIDERIS.

These studies on the pathogenicity of different pythiaceous organisms on various agricultural plants were conducted to determine the pathogenic population of a soil by means of some very susceptible plant (''indicator plant'') and to ascertain the contribution of other plants, through their susceptibility or resistance, to the increase or decrease of such a population.

The organisms used for the inoculation of the various plants had been mostly isolated from pineapples, and in few cases from such plants as spinach, snapdragon, *Carica papaya,* and castor bean. The pythiaceous organisms numbered nine species of Nematosporangium, ten of Pythium, five of Phytophthora, and one sterile Phycomycete. The hosts were planted in sterile soil in root-observation boxes. When their roots were about three to four inches long they were inoculated with pure cultures of the different fungi. The susceptibility of the various hosts was as follows: Dicotyledonous plants such as *Canavalia ensiformis* (Jack bean), *Vigna sinensis* (cowpea), *Phaseolus aureus* (Mung bean), *Vicia faba, Cajanus indicus, Helianthus annus, Ipomoea batatas, Solanum tuberosum,* and *Ricinus communis,* were susceptible to most species of Pythium and some slightly to Nematosporangium; whereas, such monocotyledonous plants as *Pennisetum barbinode, Saccharum officinarium, Zea mays, Triticum vulgare,* and *Ananas sativus* were found susceptible only to the species of Nematosporangium. Onion plants have been found very resistant to all species of Nematosporangium and Pythium, but very susceptible to all five species of Phytophthora.

Studies on the nature of host resistance to Armillaria mellea.—HAROLD E. THOMAS.

The mode of entrance and subsequent development of *Armillaria mellea* in various susceptible and resistant roots and tubers, including *Juglans regia, Prunus persica, Prunus cerasifera, Pyrus communis, Daucus carota, Pastinaca sativa, Dahlia* sp., and *Solanum tuberosum,* have been investigated.

Invasion of the root is accomplished by direct penetration of a branch of the parent rhizomorph through the sound healthy cork layer of the host. The method is similar in the several hosts investigated with no apparent difference between susceptible and resistant ones. The branch penetrates as a unit and was never observed to send

out single hyphae into the root ahead of it. In susceptible roots, after gaining entrance, rhizomorphs grow rather rapidly and cause general destruction of the host tissue. In resistant roots the fungus readily gains entrance but is ordinarily unable to establish itself. It destroys but little of the attacked root. The wounds thus formed soon cork out or heal over.

The fungus grows well on the expressed sap of certain roots and not at all or only very poorly on others, but there appears to be little correlation between the inhibition of growth and the resistance of the host.

Structural or morphological differences of the host probably exert little influence on resistance, which appears to be of the nature of a vital, antagonistic interaction between host and parasite.

Inheritance of resistance to barley scald.—W. W. MACKIE.

Barley scald is caused by *Rhynchosporium secalis* (Heinsen) Davis. The fungus survives in the soil and in diseased plant material. The spore, falling upon the leaf, germinates and pushes out hyphae which form appressoria in the stomata. Large lesions with white centers soon appear, hence the name "scald." Several generations occur during the growing season, October to March. Late-sown grain escapes attack; early-sown grain usually suffers severely. Few resistant and no immune varieties were found. Repeated crossing of highly-resistant varieties produced highly-resistant and scald-free plants under heavy attack. By crossing scald-free plants with highly-susceptible plants the following Mendelian ratios were secured for 523 F_3 families at Davis:

Scald resistance in F_3 families of hybrid barley

	Homozygous resistant	Heterozygous susceptible and homozygous susceptible
Observed	126.00	397.00
Expected	130.75	392.25
Deviation	−4.75	−4.75
Probable error	5.82	5.82
D/PE	.82	.81

The homozygous-resistant families were easily separated, for resistance is recessive. The heterozygous-susceptible and homozygous-susceptible families were difficult to separate owing to the presence of other leaf-attacking fungus diseases including mildew, spot blotch, rusty blotch, and stripe. The results indicate a 3:1 ratio of susceptibility to resistance due to a single factor for resistance.

Further studies of the modification of sugar-beet curly-top virus by its various hosts.—C. F. LACKEY.

Further investigations on attenuation of the curly-top virus by various hosts show that other wild and cultivated hosts attenuate the virus in a manner similar to that recorded in previous reports. The meaning of attenuation as here treated is the lessening of the virulence or infection power and the development of severe symptoms. In addition to the virus-attenuating plants previously reported, the following hosts have been shown to exercise this effect on it: Lamb's quarter, *Chenopodium album* and *C. album viride*; tomato, *Lycopersicon esculentum*; peasant's tobacco, *Nicotiana rustica*; hubbard squash, *Cucurbita maxima*; watermelon, *Citrullus vulgaris*; spinach, *Spinacia*

oleracea, and resistant sugar beets, *Beta vulgaris*. Fragmentary evidence indicates that Russian thistle, *Salsola kali tenuifolia*, *Kochia scoparia*, and beans, *Phaseolus vulgaris* also attenuate it. In experimental tests the mild symptoms on the sugar beets to which the attenuated virus was transferred, as well as a reduction in percentage of infection, demonstrated that the virus had been attenuated.

Several preliminary tests gave evidence that chickweed, *Stellaria media*, very susceptible to curly top, can, under certain conditions, reactivate the attenuated virus almost to its original virulence.

Hydrogen-ion concentration studies on Penicillium italicum and P. digitatum.—RAIMUND H. MARLOTH.

In the course of an investigation of the effect of sodium bicarbonate on *Penicillium italicum* and *P. digitatum*, both molds of citrus fruits, the latter being almost specific, their behavior with regard to the hydrogen-ion concentration of their media has been such as to warrant this preliminary report.

All the media used had sucrose as a source of carbohydrate. In utilizing the sucrose, the fungi produced oxalic acid, because of an incomplete respiratory process. As a consequence, the pH of the media was lowered, the degree of lowering being dependent on the buffer system. Not being a germination factor, it was found that spores of *P. italicum* showed a small percentage of germination at pH 1.9, while those of *P. digitatum* did not germinate below pH 2.3. The optimum range of pH for the former fungus for the modified Duggar's Solution was 3.0 to 7.0 and 3.5 to 8.0 for the latter.

Using the apparatus described by the writer in *Science* (LXIX No. 1794) the fungi were grown for nine days on a well-buffered medium, potassium citrate being used as buffer and the pH being kept constant by daily renewal of the solution. At the lowest growth limits, 2.0 for *P. italicum* and 2.4 for *P. digitatum*, no spores formed and the mats were fragmentary and brittle. At the neutral point and above, the mats were somewhat slimy, and those of the latter fungus formed below the surface of the solution. The optimum pH range for growth, as determined by the dry weight of the mats, was 3.0 to 5.5 for the latter and 2.5 to 6.7 for the former fungus. These ranges are subject to slight extension on the upper limit. The point brought out by these few results is the marked difference in the physiology of these two morphologically closely-related fungi. The rate of growth of the mat, as measured by the rate of oxalic acid formation, is constant once the young mat has covered the surface of the solution, and the amount of increase in weight of the mats per day, growing at different hydrogen-ion concentrations within the optimum range, is virtually the same.

Taxonomic studies in the family Pythiaceae.—C. P. SIDERIS.

The genera Nematosporangium, Pythium, and Phytophthora are differentiated by the differences in their zoospore-producing organs. There are three morphologically different organs, the prosporangium, emission collar, and zoosporangium. The prosporangium is a reservoir of the protoplasm for the development of zoospores; the zoosporangium, for the full development and discharge of zoospores. Separating the prosporangium from the zoosporangium is the emission collar through which the protoplasm passes. The wall of the prosporangium is continuous and identical with the exterior wall of the hypha supporting this organ; whereas, that of the zoosporangium is not, but constitutes a part of the so-called ectoplast or protoplasmic membrane of the prosporangium. The zoosporangium is of short duration; it emerges from the emission collar almost simultaneously with the flowing protoplasmic contents of the prosporan-

gium and lasts until the zoospores are completely formed and have escaped in the surrounding medium.

The different genera may be differentiated on this basis, as follows:

Nematosporangium: Prosporangia not well defined, nematoid, alantoid, or rarely subspherical, intra- and extra-marginal; emission collar very long; zoosporangia spherical, size variable; zoospores few to many.

Pythium: Prosporangia well defined, spherical to ovoid, mostly extra-marginal, rarely intra-marginal; emission collar short; zoosporangia spherical, size variable; zoospores few to many.

Phytophthora: Prosporangia well defined, lemon-shape to spherical, mostly extra-marginal of different sizes; zoosporangia developing within the walls of prosporangia; emission collar entirely lacking or rarely slightly developed; zoospores few to many.

Species of the genus Nematosporangium are differentiated by their rapid or slow production of oospores in culture media and tissues of hosts, by the filling or not filling of the oogonium by the oospore, and by other characters.

The species of the genus Pythium comprise four sections: (1) Those reproducing asexually, *i.e.*, zoospores and conidia; (2) those reproducing sexually, *i.e.*, oospores and conidia; (3) those reproducing sexually and asexually; and (4) those reproducing by means of conidia alone. The new sections proposed are, respectively, Zoosporium, Oosporium, Amphisporium, and Conidiosporium.

A similar subdivision of the genus Phytophthora is proposed.

A filterable microorganism from Eutettix tenellus and the sugar beet, both infected with curly top.—OLIVE SWEZY and HENRY H. P. SEVERIN.

Rickettsia-like microorganisms were found to be a common inhabitant of the intestinal tract of *Eutettix tenellus* (Baker), the carrier of curly top of sugar beets. When the insects were macerated in a suitable culture medium and passed through fine (W) Berkefeld filters, similar organisms appeared by the second or third day in the cultures made from the filtrate. No organisms have been found associated with this disease in the sugar beet. When the juice from macerated beets was passed through fine (W) Berkefeld filters and cultures made from the filtrate, organisms were obtained similar to those in the cultures from the insect. Slides were made in both cases from the filtrate immediately on passing the filter. These were fixed and stained by the same methods used with slides from the cultures but these showed no visible organisms. Cultures made from the noninfective beet leaf hopper and sugar beets gave negative results in all cases, though the same culture media were used and identical processes followed throughout. It thus appears that the microorganisms present in the infective beet leaf hopper have a filterable stage in their life history and can not be obtained from cultures from the noninfective insect or from healthy sugar beets.

The toxicity of some commercial dusts to conidia of Bremia lactucae.—JAMES B. KENDRICK.

Laboratory studies were undertaken in the autumn of 1927 to determine the efficiency of some of the common commercial dusts in preventing the germination of conidia of *Bremia lactucae*. Five sulphur compounds (Kolodust, Koloform, Dry Mix, Super Sulfodust, Powdered Sulphur) and four copper lime dusts (D-6, D-18, D-25, Copodust) were used in these tests. Conidia were secured from young lesions on lettuce leaves and a spore suspension was made in tap water. A drop of this suspension was placed on each of a series of sterile microscopic slides which had been previously dusted by means of small hand dusters. The slides were placed in Petri-dish moist chambers and held from 12–18 hours at temperatures ranging from 4°–25° C. Conidia germinated

by the production of a germ tube. The results showed that little if any toxic effect was exerted by the sulphur compounds, while in no case did germination take place in the presence of the copper-lime dusts.

Progress in the study of certain diseases of the avocado.—WM. T. HORNE.

The author has given attention to avocado diseases at the Citrus Experiment Station since July, 1928.

Carapace spot (new name) is suggested for certain large surface blemishes of mature fruit. Ordinarily, the quality of the flesh is not impaired. Preliminary evidence is given that the cause is desiccation or very slight surface abrasions. Very young fruit is involved. The study is being continued to determine particularly the effect of injuries received at different stages of development of the fruit. A parasitic etiology is not indicated.

End spots, the name given by Dr. Coit to a decline of extensive areas at the blossom end, apparently is a collapse associated with maturity and stress of climatic conditions. Neither citrus blast nor anthracnose seems ordinarily involved.

Sun blotch, apparently a chimera, is being studied with E. R. Parker, through grafting, and as to possible infectiousness.

Diseases concerned with salt relation in the soil are being studied by A. R. C. Haas, and the field aspects of his results are receiving attention. *Chlorosis, tip burn,* and *little leaf* apparently are comparable with these diseases in Citrus.

Studies of a large collection of fungi and bacteria isolated by C. C. Lindegren and H. S. Fawcett are not yet ready for report.

Further studies on Penicillia of citrus.—L. J. KLOTZ.

Histological study of citrus fruit decayed by *Penicillium digitatum* and *P. italicum* has thus far revealed no microscopic differences in the methods of attack by these organisms. The hyphae were tortuous, ramifying, ranging from 3 μ to 17 μ in thickness, distinctly intracellular as well as intercellular, and without definite haustoria; some hyphae showed very apparent constrictions at points of wall penetration. The fungi penetrated all cells of the albedo, flavedo, and core and the cells of the oil glands, but avoided the space of the glands containing oil. With advancing decay juice vesicles became involved, first the stalk cells of the vesicles and finally those cells in the expanded, juice-bearing part showing hyphae. The aerial, spore-bearing hyphae arise from heavy-wall mycelium which collects substomatally and ruptures the epidermis. Advance in action, if present, could not be detected by isolation methods, but stained microscopic sections gave an indication of such action, the affected area having no mycelium extending more than .3 mm. beyond the region bearing hyphae. Preliminary observations indicated that diastase production by *P. italicum* increased with rising temperature up to 32° C., which is near the maximum for growth. Temperature had little effect on production of invertase in either fungus.

Observations on powdery mildew of raspberry in California.—LYSLE D. LEACH.

In the fall of 1928, while studying the life history of powdery mildew of raspberries in the Santa Clara Valley, abundant perithecial formation was observed on the Ranere variety. Perithecia were present on infected canes near the apex, leaves, leaf petioles, and flower buds. Preliminary morphological studies of the perithecia, reticulations, asci, and ascospores of the causal fungus agree closely with the descriptions given for *Sphaerotheca humuli* (DC.) Burr.

To determine if perennation of the mycelium within the buds, as has been reported from Minnesota, was the common method of overwintering in the Santa Clara Valley,

four plants and 192 cane tips which had previously shown severe mildew were transferred to the greenhouse in the dormant stage. Of the 969 shoots which subsequently developed, only one showed mildew. Field observations in the spring of 1929 showed that virtually all early infection was confined to the new growth produced from the roots during the current season. The predominating appearance of the diseases on the young shoots just out of the ground suggests that the perithecia on the debris around the base of the plants may be the common source of overwintered spores in this region.

Susceptibility of cowpea varieties and selections to Fusarium wilt and root knot.—JAMES B. KENDRICK.

Serious losses are annually incurred by cowpea growers in the San Joaquin Valley, California, from Fusarium wilt and root knot. In 1927, 74 plant selections were made in a field of Blackeye cowpeas in which 90 per cent of the plants were dead from wilt and root knot. These selections and 25 varieties of cowpeas were planted in a two-acre plot in this field in 1928. Thirty-two of the selections proved to be 100 per cent susceptible. The remaining 42 selections varied in susceptibility from 55 to 95 per cent. Thirty seemed to possess some resistance and seed was saved for further tests.

The following varieties showed no evidence of disease: Brabham, Catjang, Columbia, Conch, Early Black, Iron, and Virginia Blackeye. Susceptibility varied in the other varieties as follows: Taylor, Dixie Queen Browneye, Progressive White, Victor, Cream Crowder, and Large Blackeye from 2 to 15 per cent; Extra Early Blackeye, Potomac, Groit, and Gallavant from 30 to 50 per cent; Whippoorwill, Arlington, Ramshorn Blackeye, Early Buff, Wood's No. 11 Blackeye, Early Red, New Era, and White Queen from 60 to 100 per cent.

A severe case of aerial "crown gall" on hot-house roses.—J. T. BARRETT.

The aerial form of crown gall has been reported on several hosts including rose. An unusually abundant infection on the Golden Ophelia variety in a glass house near Oakland has been under observation since December, 1928. The plants were on their own roots, had been in the beds two summers, and were large, thrifty, and productive. Practically every plant of the more than three thousand bore aerial galls. These ranged in size from a few millimeters to three inches in diameter and could be found on stems of all sizes and even on leaf petioles. Many infections took place at the out ends of the stems after removal of the flowers. Inoculations with the isolated crown-gall organism, *Bacterium tumefaciens* S. & T., are developing galls. In May of this year the plants were removed from the beds and burned. As a whole the roots were clean and healthy. Only a very few showed any crown-gall infection. In contrast, the Russell variety in another house has no aerial galls but considerable infection on the crown. The Mensing, a variety said to be closely related to the Golden Ophelia, is developing considerable aerial gall at the present time. Overhead sprinkling probably spreads the organism. No satisfactory explanation can be offered at the present time for lack of root infection on the Golden Ophelia.

Pythiaceous root parasites of pineapples.—C. P. SIDERIS and G. E. PAXTON.

Microfloral isolations from the diseased roots of pineapples yielded eight species of Nematosporangium, five of Pythium, and two of Phytophthora. The most predominant were two species of Nematosporangium, the others having been isolated only a few times, except *Pythium splendens*.

A composite soil sample of about one-half cubic foot was taken from the area under investigation, placed in a root-study box, and planted to pineapple plants. The roots were examined daily and those that died were removed and planted in Carica papaya-agar media. All of the organisms thus isolated reproduced the disease upon reinoculation.

Histological studies show that the different species of Nematosporangium may be identified inside the tissues of the host by such morphological structures as prosporangia and oospores; some species produce the latter structures readily; others slowly, or never, depending on the amount and quality of food they can obtain from the tissues. The species of Pythium may be identified by the size and shape and the smooth or spiny surface of their oospores, also by the filling or nonfilling of the oogonium. Different species of Pythium produce hues of bluish green on the diseased area which may partly serve for their identification.

Pineapple root rots caused by species of Fusarium.—C. P. SIDERIS.

About 30 species of Fusarium have been isolated from diseased pineapple roots during investigations concerned with the pathogenes involved in the wilt disease of pineapples. Few of these have been employed for the inoculation of plants grown in sterile soil. In a few cases one of these organisms has been observed invading the water-conducting vessels and killing the tip of a few of the inoculated roots, but not all, to an extent of two inches. The organism is an extremely weak parasite. The roots that had been invaded by the organism were those surrounded by very wet soil, whereas, those that happened to be in drier areas resisted invasion.

Another species of Fusarium has been observed to attack the main root through tiny rootlets or lateral roots, the resistance of which probably is low. Once it has become established on dead superficial tissue it kills the root by means of toxins. The toxin-permeated tissue becomes brown with material resulting from the dead protoplasmic contents of the pineapple root cells. The fungus never has been found among the brown dead tissue, but spreads throughout after the brown material undergoes further chemical decomposition.

Stem rot of pineapple plants.—C. P. SIDERIS.

Three pythiaceous fungi have been found causing this disease, namely, *Phytophthora meadii* and *P. melongeae* and another of possibly a new but closely related genus. *P. meadii* has been isolated from diseased plants grown on the island of Oahu and *P. melongeae* on Lanai. The disease occurs more often during the winter and spring months. *P. meadii* has been found also to cause fruit rot and *P. melongeae* stem rot of Antirrhinum.

The undetermined organism shows morphological similarity to *Pythium splendens* and *Phytophthora parasitica*. Its spherical to subspherical conidia have never produced zoospores, nor have oospores ever been observed by means of which a definite identification could be established. This organism is very destructive to young pineapple plants during periods of high humidity and low (for Hawaii) temperatures, such as 60–70° F. It may cause stem rot or root rot.

The various organisms invade the stem through the basal tissues of tender leaves and cause a soft rot.

In the case of stem rot by *P. meadii* and *P. melongeae* spraying with some fungicide may prevent the further spread of the disease. With the undetermined organism a similar treatment is not very promising as the organism is capable of living in the soil and invading the stem through the roots.

Heterothallism in Puccinia graminis.—R. F. ALLEN.

A sporidium of *Puccinia graminis* on a barberry leaf germinates promptly, passes through the outer wall, and forms a 3- to 5-cell primary hypha in an epidermal cell. Branches from this pass down into intercellular spaces. The mycelium is haploid. About

the fourth day, hyphae grow up beneath the epidermis, initiating pycnium formation. Details of the development of the pycnium and its spores have been traced.

In isolated infections, structures resembling aecia form at the normal time and place, but they consist of haploid cells only, and after undergoing some expansion and the first differentiation into areas, the whole structure dies. Usually no spores form, but there is evidence that a few old haploid aecia develop spores irregularly.

After pycniospores of different infections have been mixed, fungous cells containing two or more nuclei can be found in the wall of the pycnium, in hyphae leading from it, in a few of the hyphae between pycnia and aecia, between older and younger aecia, and beyond the youngest. Diploid cells are scattered throughout the aecium at all stages of its development and, at the time of spore formation, the centrally located diploid cells develop, initiating spore chains directly. (Cooperative investigations by the College of Agriculture, University of California, and the Bureau of Plant Industry, N. S. Department of Agriculture.)